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Alternatives in beef nutrition: Use of alternative forages and the improvement of feed efficiency on meat tenderness attributes

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DEDICATION

To my beloved fiancé Kori Trescher, my family, especially my parents Michael & Christine Blank, siblings Amanda, Roman, and Michelle, nephews Colton and Mason, Steve and Annette Trescher, and Ron & Cathy Gundrum....

... for your unwavering words of encouragement, advice, and inspiration.

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NOMENCLATURE

ADF	Acid detergent fiber
ADG	Average daily gain
ATP	Adenosine triphosphate
BAA	Beta-adrenergic agonists
BF	Back fat
BW	Body weight
Byp.	By-product
Ca^{2+}	Calcium
CP	Crude protein
d	day
DM	Dry matter
DMI	Dry matter intake
DP	Dressing percent
DRC	Dry rolled corn
EE	Ether extract
eNDF	Effective neutral detergent fiber
FCR	Feed conversion ratio
FE	Feed efficiency
F:G	Feed to gain
FP	Finishing phase
G:F	Gain to Feed
GP	Growing phase

HCW	Hot carcass weight
HFE	Highly feed efficient
HP	Heat production
ISU	Iowa State University
ISU-Corn	Finishing phase corn-based diet
ISU-Byp	Finishing phase by-product based diet
KPH	Kidney, pelvic, heart fat
LD	Longissimus dorsi
LM	Longissimus muscle
LFE	Lowly feed efficient
MEI	Metabolizable energy intake
MS	Marbling score
MU	University of Missouri
MU-Corn	Growing phase corn based diet
MU-Rough	Growing phase roughage based diet
NDF	Neutral detergent fiber
NEg	Net energy for gain
NRC	National Research Council
OM	Organic matter
QG	Quality grade
RE	Roughage equivalent
REA	Ribeye area
RFI	Residual feed intake

SBM	Soybean meal
SD	Standard deviation
SM	Semimembranosus muscle
SSF	Slice shear force
SSM	Supraspinatus muscle
SS	Sorghum silage
TDN	Total digestible nutrients
USDA	United States Department of Agriculture
WBSF	Warner-Bratzler shear force
YG	Yield grade

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"Once a man has made a commitment to way of life, he puts the greatest strength in the world behind him. It's something we call heart power. Once a man has made this commitment, nothing will stop him short of success."

~ Vince Lombardi

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ABSTRACT

Beef cattle are able to utilize high fiber feedstuffs to produce a high-quality end product for consumers; however, beef cattle are at a disadvantage relative to swine and poultry in their ability to readily convert feed resources to lean tissue. Therefore, enhancing feed efficiency (**FE**) in beef cattle is at the forefront of the industry. Biological mechanisms explaining individual animal variation in FE are poorly understood, but differences among proteolytic systems are hypothesized to play a role in this variation. Because these systems are critical to postmortem muscle fiber degradation and increases in tenderness as beef ages it is important to understand the implications for selection for more FE cattle on ultimate tenderness of beef. Additionally, little is known about how cattle diet composition impacts steak tenderness and diet components often vary widely across geographical location. Cattle producers are able to utilize waste byproducts from production of fuels such as ethanol, but they require an understanding of the feeding value of the product to safely utilize these affordable feedstuffs. Thus, the subsequent trials were designed to: 1) determine the influence of FE on meat tenderness attributes of beef steers, and 2) evaluate the use of post-ethanol extraction sorghum silage as an alternative forage in growing and finishing diets on steer performance, carcass characteristics and nutrient digestibility. Within the initial research objective, growing phase FE classification was determined from a growing phase (**GP**) residual feed intake (**RFI**) test where cattle were fed whole shell corn (**MU-Corn**) or roughage based (**MU-Rough**) diets. Within each growing phase diet the 12 greatest (**HFE**; average RFI – 3.33 ± 0.77, SD) and 12 least (**LFE**; average RFI 2.90 ± 0.94, SD) efficient steers were selected (48 steers total), and transitioned to a corn (**ISU-Corn**) or by-product (**ISU-Byp**) based diet for finishing. Growing phase diet and FE classification elicited no

difference for d 2 troponin- T degradation or calpain 1 autolysis; however, d 14 troponin-T was affected by finishing diet with greater amounts of protein degradation in steaks from steers fed ISU-Corn vs. ISU-Byp. Calpastatin activity measured on d 2 tended to be greater in HFE steers than LFE steers. An interaction between ISU diet and FE classification was noted for steak lipid content and marbling score with greater lipid in steaks from LFE vs. HFE within ISU-Byp finished steers. Measurements of beef tenderness, using the Warner-Bratzler shear force (**WBSF**) method, indicated that steers grown on a roughage-based diet (MU-Rough) had greater WBSF than steers grown on corn (MU-Corn). It is improbable that differences in WBSF were obtained from differences in ADG during the GP because ADG across FE classifications were similar for both MU-Corn and MU-Rough (1.94 kg/d) fed steers. The influence of finishing period feed efficiency (G:F) on meat tenderness attributes was determined fitting a slope regression model. Slope estimation for d 2 calpastatin and d 2 troponin-T differed from zero only for LFE steers receiving MU-Corn:ISU-Byp, indicating as G:F increases for steers receiving this respective diet there is a noted decrease in calpastatin activity with a subsequent increase in troponin-T degradation. Interestingly, the slope for d 14 troponin-T of HFE steers receiving MU-Rough:ISU-Corn differed from zero, suggesting as finishing phase G:F increases there is a decrease in troponin-T degradation. Within the second research objective, total tract apparent digestibility of organic matter (**OM**), ether extract (**EE**), crude protein (**CP)**, and neutral detergent fiber (**NDF)** were greater, and acid detergent fiber (**ADF)** digestibility was decreased for steers fed diets containing the post-ethanol extraction sorghum silage relative to a medium quality grass hay; however, no differences were noted for the digestibility of DM and starch. Although growing phase digestibility was different, overall steer performance and carcass characteristics were

not affected by the inclusion of post-extraction sorghum silage in feedlot diets, thus suggesting post-ethanol extraction sorghum silage may be utilized effectively as an alternative forage source in feedlot diets. These findings provide opportunity for a new forage by-product feed to be incorporated into feedlot diets and suggest that protein metabolism is influenced by FE phenotype, though the importance in explaining biologic variation in FE remains to be elucidated. Additionally, this research has revealed interactions among FE phenotype and cattle diet composition and further research is warranted to better understand the implications of nutritional management across different FE classifications, and the alterations in protein expression that may lead to differences in beef tenderness.

CHAPTER I

GENERAL INTRODUCTION

Considerable variation in feed efficiency (**FE**) exists amongst livestock species, and perhaps more importantly, individual animals within a species may vary tremendously (Koch et al., 1963). Although our understanding of the physiological mechanisms altering FE is limited, it has been hypothesized that changes in protein turnover, tissue metabolism and stress provide a large contribution to the variation in feed efficiency (Herd and Arthur, 2004). Protein turnover and tissue metabolism *in vivo* and postmortem are under the control of many different systems with varying activities. Those with minor roles in protein metabolism include systems such as the cathepsins, lysosomal, and proteasome, but the primary contributor is the calpain system. The calpain system contains a series of Ca-dependent proteases (calpain 1 and 2) that function to degrade protein, as well as the potent endogenous inhibitor calpastatin, which serves as the primary regulator of protein turnover (Goll et al., 1998). Increased calpastatin activity has been associated with decreases in protein degradation, thus increasing net protein accretion. Selection for improvements in FE in cattle and pigs may result in greater calpastatin activity as highly efficient animals have greater calpastatin activity (McDonagh et al., 2001; Cruzen et al., 2013); however, high calpastatin activity may result in the production of less tender meat products (Shackelford et al., 1991a; Goll et al., 1998). Additionally, the most impactful organoleptic property for consumer perception of meat products is tenderness; however, the associations between ruminant diet and ultimate meat tenderness are inconclusive.

In ruminant species, dietary roughage is important for the maintenance of rumen health, decreasing the likelihood of metabolic challenges, like acidosis, to optimize cattle performance.

Considerations of roughage inclusions late in the feeding period and the effects on cattle performance have been evaluated, producing a concept known as energy dilution. Energy dilution results from increased concentrations of roughages in high concentrate finishing diets, resulting in lower dietary NEg, to which ruminants respond by increasing DMI in order to maintain energy intake (Galyean and Defoor, 2003). Thus, increasing DMI to maintain similar energy intake for gain lessens efficiency of feed utilization. Therefore, roughage concentrations in U.S. feedlot diets during the finishing phase are typically between 8-10%, depending on forage quality (Samuelson et al., 2016). Advances in processing methods used by the ethanol and other industries have increasingly been able to extract soluble fiber fractions for industrial uses, altering the quality of forages, potentially affecting the way processed forages are utilized in feedlot diets.

The research presented in this thesis is sought to answer two questions driven by recent changes in the beef and ethanol industries: 1) does the FE phenotype of cattle impact tenderness of beef and 2) what impact does the ethanol extraction process have on the nutrient availability of sorghum silage when fed to feedlot cattle.

Thesis organization

The following chapter (Chapter 2), provides a detailed review of the literature related to the topics of the major and minor systems involved in protein metabolism, the effects of residual feed intake (RFI) on energy metabolism, carcass and body composition, and protein turnover, followed by consumers' perception and measurements of beef tenderness, and concludes with a review of nutritional management in beef production with particular focus on the role of roughages in ruminant diets. The next two chapters present research that has been completed

related to these subjects, including manuscripts to be submitted to the Journal of Animal Science. Chapter 3 contains research conducted to evaluate the influence of feed efficiency on meat tenderness attributes of beef steers. Chapter 4 is focused on the use of post-ethanol extraction sorghum silage as a forage source in growing and finishing feedlot diets and the effects on steer performance, carcass characteristics, and nutrient digestibility. Finally, Chapter 5 contains general conclusions of overall research findings and suggestions for future research.

CHAPTER 2.

REVIEW OF THE LITERATURE

Minor Systems Involved in Protein Metabolism

Caspase system

The caspase system is a class of Cys proteases that function without the presence of Ca to degrade proteins during apoptosis, or cell death, similar to the fate of skeletal muscle following exsanguination when nutrient and oxygen transfer ceases (Goll et al., 2008). Important components of this system are caspase 9, 3, and 7. Caspase 9, known as the initiator caspase regulates the mitochondrially gated pathway of apoptosis; however, caspase 3 and 7, commonly referred to as the executioner caspases which are activated by caspase 9, cleave the protein substrates resulting from apoptosis (Earnshaw et al.,1999). Kemp et al. (2006), using Large White gilts (81.2 ± 1.98 kg) collected longissimus samples at 0, 2, 4, 8, 16, 32, and 192 h post-harvest for determination of the role caspases have in meat tenderization, measuring alpha II spectrin as an indicator of degradation due to caspase activity. The authors noted a positive correlation of alpha II spectrin to caspase 3/7 ($r = 0.38$) and caspase 9 ($r = 0.32$). In addition, a negative relationship was observed between caspase activity ratio and shear force (caspase 3/7: $r = -0.62$; caspase 9: $r = -0.68$), as well as shear force and alpha II spectrin (120 kDa) degradation product ($r = -0.75$), suggesting as caspase activity increases WBSF is decreased indicating the potential for a more tender product. A later study by Kemp et al. (2009) examined the effect of aging on activity of the caspase system using longissimus muscle samples (aged 0, 4, 8, and 24 h, and 2, 7, and 21 d), as well as semimembranosus, and infraspinatus muscle samples (aged 0, 8, and 24 h, and 7 d) from 24 lambs (12 callipyge, 12 noncallipyge) 14 months of age. Activity of

all three caspases decreased overtime for callipyge and noncallipyge lambs, with a faster decline noted for caspase 9 than caspase 3 and 7. Interestingly, these authors reported a negative relationship between peak caspase 3 and 7 activity at 8 h in normal lambs and calpastatin activity measured at d 0 and 2 (r = -0.65 and r = -0.68); however, this was not observed in callipyge lambs, to which they speculate in normal lambs caspase 3 and 7 may be cleaving calpastatin; however in callipyge lambs the calpastatin activity is so great that caspase 3 and 7 cannot degrade it sufficiently, thus, supporting the greater protein accretion observed in callipyge lambs. In a study using Jurkat cells (human leukemic T-lymphocytes) and human myeloid leukemic lymphocytes incubated with cell lysates and purified enzymes. Porn-Ares et al. (1998) noted that recombinant caspase-3 cleaved the calpastatin protein within 4 h of incubation. A series of three experiments conducted by Underwood et al. (2008) examined the effects of caspase activity on beef tenderness. Evaluating caspase activity over time in Exp 1, the authors used longissimus thoracis and sternomandibularis muscle samples collected at 0, 0.25, 1, 3, 24, 72, and 240 h postmortem from 5 crossbred steers, noting a decrease in caspase activity at 72 and 240 h. For Exp. 2 a subsample of carcasses were selected from 5 crossbred steers each with low WBSF (3.77 ± 0.20 kg) or high WBSF (4.44 ± 0.18 kg) from a larger group (n = 40) to determine caspase activity. In Exp. 2 a lesser amount of caspase 3 activity was noted in low WBSF carcasses compared to high WBSF carcasses. Lastly, for Exp. 3 a subset of steers (n = 5) matched for growth characteristics were selected for low WBSF (3.60 ± 0.18 kg) and high WBSF (5.48 ± 0.12 kg), and no differences in caspase 3 activity were noted between high and low WBSF groups with similar growth characteristics. Relative to systems such as the calpains (discussed later), it appears the caspase system plays a minor role in protein degradation in beef cattle and may have limited effects on postmortem tenderness of beef. However, more work is

needed to clarify the importance of even minor protein degradation systems in explaining biological differences among cattle of varying feed efficiency.

Lysosomal system (cathepsins)

Lysosomes, found in small quantities within skeletal muscle contain Cys proteases known as cathepsins which function within an optimal pH range of 3.5 – 6.0. Because this pH range is below that of the cell cytoplasm cathepsins become activated by the low pH of the lysosome. Lysosomes are smaller in size than the myofibrils (0.5 to 3.0 μm), which limits their ability to degrade myofibrillar protein typically resulting in the severing of the myofibril and loss of function (Goll et al., 2008). Expression of cathepsin is greatest in tissues with high rates of protein turnover (liver, kidney, spleen, placenta) and is lesser in skeletal muscles, and the major cathepsins include: cathepsins L (endopeptidase), B (carboxypeptidase), D (aspartic endopeptidase), and H (aminopeptidase; Bechet et al., 2005). In a comparison of Bos indicus cross (Sahiwal) versus Bos taurus (Hereford x Angus) steers and heifers for the determination of cathepsin B and B+L activity, Whipple et al. (1990) used 5 g of longissimus muscle collected at 1 and 14 d postmortem. The authors observed no effect of breed composition on cathepsin B or B+L activity at either time point, suggesting that cathepsins have minimal effects on the postmortem protein degradation of beef. Johnson et al. (1990) evaluated the effect of total cathepsin B and L activity in steers (n = 32 total) of known breed compositions Angus (A) and Brahman (B), (100% Angus; ¾ A × ¼ B; ½ A × ½ B; ¼ A × ¾ B) on meat tenderness. Results of this study showed greater amounts of cathepsin B and L activity in carcasses from Angus steers than Brahman cross steers. A general trend was observed that as the percentage of Brahman increased there was less cathepsin activity and increased WBSF measured on d 10 resulting in a

negative correlation between cathepsin B and L activity and WBSF (r = - 0.441). Utilizing Charolais bulls (n = 8) and steers (n = 7) 15 months of age Calkins and Seideman (1988) evaluated the effects of cathepsin B and H (determined at 1 h postmortem) on meat tenderness and changes that occur during postmortem aging. Determination of WBSF was conducted on LM on d 1, 3, 6, 9 , and 14, results were pooled and no difference was detected between bulls and steers; however, overall change in WBSF was positively correlated with total activity of cathepsin B (r = 0.44) and H (r = 0.64). Activities of cathepsin B and H collectively contributed approximately 35 and 58% of the variation in change of WBSF from d 1 to d 14 and from d 3 to d 6, respectively (Calkins and Seideman, 1988). These data suggest cathepsins have a role in early postmortem protein degradation due to the rapid increase in lactic acid, decreasing the pH enough to activate the cathepsins, before tapering off as muscle reaches an ultimate pH between 5.4 and 5.6.

The proteasome

The 26S proteasome functions in ATP-ubiquitin dependent proteolysis; however, when ATP is depleted the 26S proteasome dissociates into the 19S regulatory unit and 20S proteasome, which has the capability to degrade protein without ATP-ubiquitin activation (Robert et al.,1999). The proteasome pathway accounts for approximately 80 to 90% of the degradation of all intracellular muscle proteins; however, due to the size of myofibril proteins and the inability to enter the central chamber of the proteasome where the catalytic residues are located, they are unable to be degraded directly, and thus are tagged by ubiquitin molecules for degradation (Goll et al., 2008). **Figure 1**, adopted from Goll et al. (2008), shows the function of the proteasome in degrading a protein, beginning with activation of ubiquitin (step 1) to the E1

ubiquitin-activating protein which is then transferred to the E2 conjugating enzyme (step 2) prior to being transferred to the target protein (step 3). After acquiring a minimum of 4 ubiquitin tags the target protein is then recognized by the 26S proteasome (step 4) and degraded into small peptides (step 5; Kemp et al., 2010), with ubiquitin molecules recycled (step 6) to activate and target another protein for degradation. Robert et al. (1999), using bovine myofibrils originating from the longissimus dorsi, evaluated the effects of the proteasome on 14-C labelled myofibrillar proteins (actin, troponin, myosin, filamin) following incubation with purified 20S proteasome for 0, 4, 24 h at 37° C. These authors concluded that myofibrillar proteins are hydrolyzed to varying degrees, in addition to degradation of non-myofibrillar proteins (albumin, casein, haemoglobin), thus, the 20S proteasome has the capability to hydrolyze myofibrillar proteins of high molecular weight. The requirement of ATP for the activation and targeting of proteins for degradation suggests the proteasomes contribution occurs early postmortem prior to the depletion of ATP when carcasses enter rigor mortis and has little contribution over the aging period.

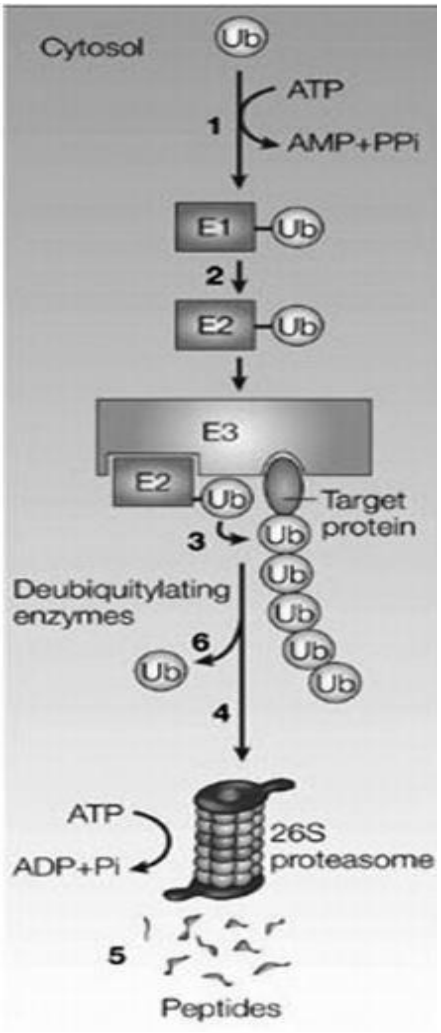

Figure 1: Ubiquitin process targeting a protein for degradation, adopted from Goll et al. (2008).

The presently discussed systems contribute variable amounts to the metabolism of protein within livestock species and during postmortem aging; however, of more practical

importance is the Ca^{2+}-dependent calpain system which makes greater contributions in postmortem protein degradation, impacting the tenderness of meat products.

The Calpain System

Role in muscle growth

The fundamental purpose of the calpain system *in vivo* is to regulate protein turnover and muscle growth. The rate of muscle development is largely influenced by differences in the rate of protein synthesis and degradation under the control of two proteolytic enzymes (calpain 1, calpain 2) and their potent inhibitor calpastatin (Goll et al., 1998). The discovery of the calpains was initiated by the work of Busch et al. (1972) using psoas strips from rabbits incubated in 1mM Ca for 9 h at 37˚C and pH 7.1, resulting in the removal of the Z-lines of the skeletal muscle myofibril structure. Later, Dayton et al. (1976) purified the Ca^{2+}- dependent proteolytic enzyme m-calpain (currently called calpain 2) from porcine skeletal muscle extracts. Follow up work by Dayton et al. (1981) identified a protease requiring lesser Ca^{2+} concentrations for activation, initially called μ-calpain, but more recently known as calpain 1. Results of an immunofluorescence assay of rat skeletal muscle suggest calpain 1 is localized in the cytoplasm of muscle cells, whereas calpain 2 is associated with plasma membranes (Schollmeyer, 1986). Calpain 1 undergoes Ca^{2+}- induced autolysis to become active, the inactive 80 kDa subunit undergoes multiple cleavages forming the 78 kDa intermediate, and lastly to the active 76 kDa subunit, having the ability to degrade protein (Zimmerman et al., 1991). The ability of calpains to undergo autolysis results in less Ca^{2+} required for activation; however, when activated via autolysis, calpain function is lost over time (Suzuki et al., 1981; Dayton, 1982). Calpain activity is thought to be regulated by the activation of the calpastatin molecule as the Ca^{2+} concentration

required to activate calpastatin is equal to, or slightly lesser than, that required to activate the calpains (Kapprell et al., 1989; Goll et al., 1992). Goll et al. (1992) reported that Ca^{2+} concentrations required for half-maximal activity of calpain 1 (2-20 μM Ca) calpain 2 (200-700 μM Ca) were much greater than free cellular Ca^{2+} concentrations (0.2-0.8 μM Ca) found under typical conditions. In a study conducted by Kapprell et al. (1989), using a calpastatin affinity column with variable Ca^{2+} concentrations, it was observed that calpain 1 required approximately 600 nM Ca for half-maximal activity, but only 42 nM for half-maximal binding to calpastatin, its inhibitor. Binding of Ca^{2+} to the calpains initiates a conformational change allowing the binding of calpastatin and the inactivation of calpain (Goll et al., 1992). Differences in Ca^{2+} concentrations required for activation of the calpains and calpastatin underpins the hypothesis of Goll et al. (1998) that calpastatin may be the primary regulator of rates of protein turnover.

One example of the impact of alterations in the calpain/calpastatin system is found in lambs expressing the callipyge gene. The callipyge phenotype in sheep is characterized by postnatal increases in muscle hypertrophy of the loin and muscles of the pelvic girdle (Duckett et al., 2000; Cockett et al., 2004). In a study using 40 lambs (20 callipyge, 20 normal) Duckett et al. (2000) harvested 4 lambs per treatment at each of several weights (7, 20, 36, 52, 69 kg) to detect differences in muscle weight (LM, SM, SS), calpastatin activity, and WBSF. Lambs expressing the callipyge gene exhibited approximately 45% greater muscle weights (LM, SM) and on average an 81% increase in calpastatin activity in the LM. Similar increases of 83% and 75% in calpastatin activity of callipyge vs. normal lambs have been documented by others (Koohmaraie et al., 1995; Clare et al., 1997). Thus, increased calpastatin activity exhibited by callipyge lambs lessens the ability of the calpains to degrade protein resulting in the net accretion of muscle.

In feedlot cattle, improved muscle growth is often accomplished through the use of β-adrenergic agonists (BAA), and increases in muscle growth may be partially attributable to effects on the calpain system. Effects of BAA on the calpain system, specifically calpastatin activity have been used to support the assertion that increased calpastatin activity plays a role in BAA- induced muscle accretion *in vivo*. Calpastatin activity responses to BAA administration have been variable across species. In a study using New Zealand white rabbits calpastatin activity was greater in rabbits which had received BAA by 52% (Forsberg et al., 1989); however, in a study by Kretchmar et al. (1990) post mortem calpastatin activity had been increased from 74% at d 0 to 430% by d 4 in the longissimus dorsi of lambs fed a BAA compared to controls (Kretchmar et al., 1990). Wang and Beermann (1988) used 28 Dorset lambs administered 10 ppm of a BAA (cimaterol) for a period of 3 or 6 weeks and reported an approximate 55% and 70% decrease in the activity of calpain 1 measured on d 2 from the longissimus dorsi (**LD**) of lambs receiving BAA treatment for 3 and 6 weeks, respectively. Collectively, an increase in calpastatin activity in combination with a decrease in calpain activity results in less protein degradation and the net accretion of muscle protein.

Postmortem proteolytic activity

Traditional harvest practices in the U.S. beef industry affect postmortem calpain system activity. After exsanguination oxygen supply to the muscle ceases and anaerobic metabolism products increase. This results in the buildup of lactic acid, causing muscle pH to decline from 7.0 to values between 5.4 and 5.6, the ultimate pH of beef. This change in pH in combination with a rapid temperature decline increases the concentration of free Ca released from the sarcoplasmic reticulum initiating the activity of the calpain system (Koohmaraie, 1992). Changes

in postmortem conversion of muscle to meat are readily attributed to the changes in the proteolytic activity of calpains 1 and 2 on key myofibrillar proteins which constitute the major protein fraction in skeletal muscle, accounting for much of the variation in tenderness of the LD (Koohmaraie, 1992, 2002). Koohmaraie et al. (1991) has suggested the ratio of activities of calpain 1 to calpastatin is approximately 1:4 in beef longissimus muscle. Greater activity of calpastatin indicates decreased calpain activity resulting in less protein degradation, potentially leading to less tender beef (Goll et al., 1998). Calpain 1 located inside the muscle accounts for approximately 55-60% of the total protein in muscle cells (Goll et al., 2008) and has a critical role in postmortem proteolysis through degradation of myofibrillar proteins. Activation of the calpains has been estimated to account for 68 to 95% of the postmortem tenderization occurring during the first 14 days of storage (Dransfield, 1992; Goll et al., 1998). Geesink et al. (2006) evaluated the importance of calpain 1 in protein breakdown using muscles dissected and snap frozen from the hind limbs of wild-type and calpain 1 knockout mice on d 0, 1, and 3 postmortem, reporting that 1 d postmortem wild-type mice had 90% degradation of protein compared to no degradation in calpain 1 knockout mice. Kent et al. (2004) reported less 80 kDa calpain 1 and desmin protein remaining at 7 d postmortem in control mice (13 and 9%, for calpain 1 and desmin, respectively) than transgenic mice over expressing calpastatin (67 and 83%, for calpain 1 and desmin, respectively). This suggests less calpain 1 activity and subsequently decreased protein degradation in the presence of its inhibitor calpastatin. The work of Geesink and Koohmaraie (1999), using bovine sternomandibularis myofibril samples incubated for 0, 1, 2, 7 and 14 d at 5° C with 0, 0.25 or 0.50 units of calpastatin, suggested that increasing calpastatin limits the rate and extent of myofibrillar protein degradation (titin) and calpain 1 autolysis. These authors further concluded after one day of incubation full autolysis of

calpain 1 was practically complete; however, myofibrillar protein degradation continues to d 7 postmortem with minimal improvements between d 7 and 14. Veiseth et al. (2001), using market weight crossbred lambs, collected longissimus lumborum samples at 3, 6, 9, 12, 24, 72, 360 h post mortem and examined the effects of storage on activity of calpain 1 and 2. They found that after 24 and 72 h of storage calpain 1 activity was decreased by 42% and 95%, respectively, with no remaining activity detected at 360 h postmortem. Results of Boehm et al. (1998) using bovine semimembranosus muscle collected from steers and heifers ranging in weights from 360 to 550 kg, concluded that 1 d post-harvest calpain 1 activity was 20% of that reported at harvest and by d 7 activity had declined to less than 4%. Interestingly, calpastatin activity was most variable with activity being decreased by approximately 17 to 67% at 1 d postmortem and 15 – 38% after 7 d. Contrary to the role of calpain 1 activity in postmortem protein degradation, it has been well documented that calpain 2 has minimal contribution to protein degradation due to insufficient amounts of free Ca available for activation (Koohmaraie et al., 1987; Goll et al., 1992; Veiseth et al., 2001).

Calpain system conclusion

Activity of the calpain system has been extensively researched under postmortem conditions though understanding of the mechanisms affecting changes in activity *in vivo* that elicit responses in animal performance remain unclear. Research evaluating post-harvest treatments in combination with storage on the activity of the calpain system has been conducted, and extreme variability in the activity of calpain components has been documented across species. Because variation in calpain system activity exists postmortem, it seems plausible that differences in calpastatin activity *in vivo* may explain some of the biological variation in FE among individual

animals. It is well understood that a greater proportion of muscle accretion occurs in the earlier stages of the growth followed by increased lipogenesis in the latter portion of the cattle feeding period. Despite understanding the response of calpastatin activity as a result of administering β-adrenergic agonists late in the feeding period to alter the ratios of fat to protein accretion, minimal work has been done to evaluate the mechanisms of activation *in vivo* throughout the growth curve which elicits the observed alterations in animal performance. Because of the limited *in vivo* work examining the calpain system it is not surprising that effects of nutritional management throughout the feeding period on calpain system activity have also not been evaluated.

Feed Efficiency

Residual feed intake

The rate at which a kg of lean is produced from a kg of feed consumed has been the historical method by which feed efficiency has been measured in the beef industry. Koch et al. (1963) introduced a new concept for the calculation of feed efficiency, commonly known as residual feed intake (**RFI**). They suggest that feed intake may be adjusted for maintenance and gain requirements by two methods: 1) that expected to achieve desired performance and 2) residual feed. Thus, animals that consume less feed than what is predicted have a negative RFI value and are classified as highly efficient, whereas a positive RFI value is indicative of a lowly efficient animal that required more feed to achieve similar gain. Herd and Arthur (2009) estimate that much of the variation in RFI is in response to differences in metabolic processes among individual steers. Despite many attributes of physiological differences amongst cattle which impact RFI, heritability has been estimated to range from 0.16 to 0.43 (Herd et al., 2003). After a

single generation of selection for post-weaning RFI in Angus feedlot steers 8 to 12 months of age, Herd et al. (2003) recorded favorable correlated changes in average daily feed intake (9.2 ± 0.2 vs. 9.8 ± 0.2 kg/d) and RFI (-0.20 ± 0.11 vs. 0.17 ± 0.10 kg/d) in comparison to steers that were not selected for RFI. In support of these positive changes Nkrumah et al. (2004), utilizing 150 crossbred steers ranging in RFI from -2.25 (highly efficient) to 2.61 kg/d (lowly efficient), noted positive phenotypic correlations between RFI and DMI (r = 0.75), metabolizable energy intake (MEI; r = 0.83) and feed conversion ratio (FCR; r = 0.62), thus as RFI decreases there is a decrease in DMI, MEI, and FCR. The directions of these correlations to RFI combined with the moderate heritability favor the selection for low RFI in future beef production.

RFI energy metabolism

The assessment of cattle performance and carcass characteristics in relation to RFI has been of recent interest in an effort to understand the biological mechanisms that may be driving differences noted in phenotypic RFI across species. Richardson and Herd (2004) and Herd and Arthur (2009) estimate that digestibility, metabolic heat production, physical activity, and differences in body composition account for 10, 9, 10, and 5% of the variation observed in RFI in cattle, respectively. Basarb et al. (2003), using 172 crossbred steers classified as high, medium, and low RFI in a serial slaughter study, evaluated the relationship between RFI, growth rate, body composition and heat production (**HP**). Steers classified as lowly efficient (high RFI) had increased deposition of empty body fat but a slower rate of protein accretion, despite a 10.2% increase in MEI. The differences in body composition may be attributed to the retention of approximately 12% more energy (as fat), and the production of 9.3% more heat than low RFI (highly efficient) steers (Basarb et al., 2003). Similar results were documented by Gomes et al.

(2012), using a subset of the 12 greatest and least efficient steers selected from a larger population of Nellore steers, in that high RFI steers required greater MEI intake for maintenance (160 vs. 131 Mcal/kg). Differences in feed intake and energy partitioning altering the body composition of steers may be supported by the conclusions of Ferrell and Jenkins (1998) in that crossbred steers fed high-concentrate diets (83% corn) *ad libitum* had greater MEI and visceral organ weights than limit fed steers. Thus, feed intake is greater than expected for BW gain to meet the energetic demands of the gastrointestinal tract and residual feed energy is deposited as fat (retained energy).

RFI carcass characteristics and body composition

Multiple studies have reported minimal to no differences in carcass characteristics (HCW, DP, REA, KPH, YG, QG) across groups of steers classified as high or low RFI (McDonagh et al., 2001; Baker et al., 2006; Gomes et al., 2012). Crossbred steers receiving growing diets (roughage or whole shell corn) and finishing diets (by-product or cracked corn-based diets) in a multi-year FE study conducted by Russell et al. (2016) noted that steers classified as highly feed efficient (HFE) tend to have a larger REA than their lowly efficient (LFE) counterparts. Similarly, the results of Smith et al. (2011), using pigs selected for decreased RFI noted a tendency for lesser amounts of BF and greater amounts of fat-free lean in highly efficient pigs. In contrast, Bonilha et al. (2013) in a study using 49 Nellore bulls classified as high and low RFI reported no differences in empty body composition (water, EE, protein, minerals, and retained energy) between low and high RFI bulls as determined by complete homogenization of the left side of the carcass. The lack of differences in the study by Bonilha is

likely a response of breed type, as it is well understood that *Bos Indicus* influenced cattle and bulls are leaner than cattle of *Bos Taurus* influence.

RFI and protein turnover

Variation in RFI related to differences in protein turnover, tissue metabolism and stress is estimated to be 37% (Richardson and Herd, 2004; Herd and Arthur, 2009). Contrasting correlations between RFI and BF gain (r = 0.30) and between RFI and protein accretion (r = -0.21) have been reported by Nkrumah et al. (2004). Thus, selection for low RFI potentially decreases fat synthesis and improves protein accretion. In a study with Angus and Angus cross cattle following a single generation of divergent selection for RFI, McDonagh et al. (2001) reported approximately 13% greater calpastatin protein in the LD of HFE animals, and low RFI cattle had lesser amounts of myofibril fragmentation indicating less protein degradation. In contrast, Baker et al. (2006) using a subsample of carcasses (n = 32) selected from a larger population of 54 purebred Angus steers classified as high (> 0.5 SD from the mean), medium (± 0.5 SD from the mean) and low (< 0.5 SD from the mean) RFI groups, assessed the relationship between RFI and performance variables and found that longissimus muscle calpastatin activity measured at 24 h postmortem was not different between high and low RFI groups. These authors further noted no differences in WBSF measured on strip loins aged 1, 3, 7, 14 and 28 d; however, WBSF did tend to decrease as postmortem aging time increased as evidenced by the negative correlation between WBSF values and postmortem aging time (r = -0.47, P < 0.0001; Baker et al., 2006). The results of Gomes et al. (2012) support those of Baker et al. (2006) observing no effects of RFI classification on WBSF and activity of calpain 1, calpain 2 and calpastatin, using a subset of the 12 greatest and least efficient Nellore steers selected from a larger population.

Cruzen et al. (2013) evaluated differences in RFI and its effects on the calpain system (calpain 1 and 2 autolysis, calpastatin activity), utilizing 12 gilts selected for 7 generations for low RFI (highly FE) and 12 selected from 2 generations for high RFI (lowly FE). These authors concluded that low RFI pigs tended to have decreased calpain 1 and 2 autolysis and greater calpastatin activity, which resulted in a decreased calpain 1 to calpastatin activity ratio, suggesting lesser protein degradation. Measurement of troponin T degradation at 3 d postmortem in loin samples indicated a slowing in the rate of protein degradation of low RFI pigs, suggesting a lesser extent of calpain 1 activation postmortem in carcasses of highly efficient pigs. In a similar study, Smith et al. (2011) reported increased d2 calpastatin activity in the longissimus muscle of barrows that had been selected for decreased RFI over 5 generations. The previously reported relationship between selection for decreased RFI and increased calpastatin activity suggests there is lesser protein degradation resulting in the net accretion of protein. Richardson et al. (2001) evaluated measures of protein accretion on 140 Angus steer progeny of parents selected for low RFI (high efficiency) or high RFI (low efficiency) concluding that highly efficient (low RFI) steers had greater protein gain than lowly efficient cattle (15.1 ± 0.59 vs. 12.8 ± 0.79 kg DM), reporting a negative correlation between RFI and protein gain (r = -0.50). A similar trend was noted in chemical protein as a percent of final BW with low RFI (highly efficient) steers having greater percent protein than high RFI (lowly efficient) steers; however, numerical trends for fat content were opposite with lowly efficient steers having greater deposition of fat (40.2 ± 3.60 vs. 36.5 ± 2.29 kg DM) and a greater percent of final BW as fat compared to highly efficient steers. Further evidence of lean tissue metabolism was documented by Richardson et al. (1996) measuring blood profiles of Angus steers and heifers ranked for high and low RFI, tested on pelleted diets of approximately 70% luceme hay: 30% wheat mixture

over 120 d period, noting a greater total plasma protein concentration in high RFI steers (70.1 ± 0.7g/L) versus low RFI animals (65.2 ± 0.7 g/L). A follow up study by Richardson (2004) reported a positive correlation (r = 0.26) between RFI and total plasma proteins of cattle at weaning. Plasma proteins as reported by Clarke et al. (1996) have an important role in the supply of amino acids for protein synthesis. A secondary blood metabolite aspartate amino transferase serves as an indicator of protein metabolism by the liver. Richardson et al. (2004) reported a positive correlation with RFI and aspartate amino transferase (r = 0.43). Thus, lesser amounts of these blood metabolites could serve as a potential indicator of animals to be more feed efficient, as a result of less protein degradation.

RFI conclusions

The relation between RFI and phenotypic variation of performance and body composition in livestock species has been well researched. Pigs and cattle alike have shown that animals with greater RFI (lower FE) require more feed and have altered metabolic pathways retaining greater amounts of energy as adipose tissue. Conversely, efficient energy utilization in livestock species supports that of lean tissue accretion, evidenced by the lesser MEI required to achieve similar gains between highly and lowly efficient livestock. The proteasomal mechanisms involved in the turnover of protein require energy; however, the greater calpastatin activity of highly efficient animals inhibits protein degradation by calpain 1 and 2, thus lessening their energy requirements making them more efficient. Improved efficiency of livestock, especially cattle, is a desired trait for genetic selection to improve profitability for livestock producers. However, as increased calpastatin activity postmortem decreases the activity of the calpains the extent of tenderization of meat is lessened, presenting concerns for consumer acceptance of meat produced from highly

efficient livestock. Further research is needed to identify biomarkers that can be used for management and selection of livestock with greater FE, aiding in the understanding of the potential effects improving feed efficiency may have on meat quality.

Beef Tenderness

Consumers' perceptions and market choices

The importance of beef tenderness to overall palatability and consumer satisfaction has been well documented (Miller et al., 1995, 2001, Corbin et al., 2014). A national beef tenderness study by Miller et al. (2001) evaluated the USDA Select strip loin steaks of known WBSF (> 5.7 kg = tough; < 3.0 kg = tender) to determine a threshold and monetary value consumers place on tenderness, utilizing 1,036 steaks aged for 7 or 21 d, and cooked to an internal temperature of 71° C. The study was conducted in three supermarkets across five metropolitan areas and steaks were evaluated by 734 consumers of diverse backgrounds. These authors reported consumer tenderness acceptability increased as measurements of WBSF decreased, concluding that tenderness thresholds of < 3.0, 3.0 to 4.3, and > 4.9 kg would satisfy customers 100, 93 and 25% of the time, respectively; with 78% of consumers willing to purchase steaks if guaranteed to be tender. Similar results are noted by Huffman et al. (1996) using loin steaks evaluated in homes and white table cloth restaurants by 67 consumers over a 15-wk. period in Lubbock, TX with a total of 739 observations. These authors concluded a WBSF value of 4.1 kg results in a 98% consumer acceptability in both home and restaurant scenarios. In 2013 the USDA launched a certified tender program with a tenderness threshold value adopted from ASTM International (2011), determined by a WBSF value of 4.4 kg or 20.0 kg slice shear force (SSF) or less for a cut of beef to qualify. Several studies have not only evaluated consumer preferences for tender beef,

but also premiums that can be attained by the beef industry for providing certified tender beef products to consumers. Using beef top loin steaks, with different WBSF values (tender = 2.27 to 3.58 kg; intermediate = 4.08 to 5.40 kg; tough = 5.90 to 5.21kg) Boleman et al. (1997) reported how consumer buying trends were influenced by tenderness and price differences. The authors concluded that overall satisfaction was greater for tender (tender: 16.91) than the other categories (intermediate: 14.06; tough: 12.90) with a similar trend noted in tenderness (tender: 16.61; intermediate: 13.66; tough: 11.61) as reported by the consumer, suggesting consumers are able to detect differences in tenderness. Despite price differentiation between categories of $1.10/kg, when given the choice, consumers purchased: 94.6%, 3.6% and 1.8% of the steaks classified as tender, intermediate, and tough, respectively. Platter et al. (2005) using beef strip loin steaks (*n* = 541) evaluated the effects changes in marbling (USDA quality grade standards) and WBSF values have on consumer (*n* = 489) behavior and willingness to purchase steaks. In comparison of prices across tenderness measurements used in this study (very tender ≤ 3.4 kg, slightly tender, 3.41 to 4.40 kg; slightly tough, 4.41 to 5.40 kg; very tough, > 5.40) steaks classified as slightly or very tough in comparison to slightly tender were discounted $1.26/kg and $1.72/kg, respectively, as well as the mean prices decreasing $1.02/kg for every 1 kg increase in WBSF. Similar differences in pricing of strip loin steaks were noted by Miller et al. (2001), recording a difference of $0.59/kg between tender (< 3.0 kg) and intermediate (> 3.0 to 4.6 kg), $1.08 between tender and tough (>4.9 kg), and $1.23/kg between tender and the toughest steaks (> 5.7 kg). Present USDA quality grades from greatest to least are Prime, Choice, Select, and Standard. Given USDA Select is typically perceived as the lowest quality beef, Shackelford et al. (2001) evaluated strip loins from USDA Select carcasses and the effect a Tender Select beef brand, defined as “the only steak guaranteed tender and lean,” has on consumer (*n* = 759) perceptions

and willingness to purchase a tender beef brand. When presented with the option to purchase Tender Select beef, 89% of consumers indicated they would consider or would buy the product, and 65% of consumers indicated that if offered they would buy all their beef cuts at a retailer that supplied guaranteed tender beef.

Measurements of meat tenderness

Objective measurements of tenderness are most commonly conducted using two methods: WBSF or Slice shear force (**SSF**). For determination of WBSF, six cores 1.27 cm in diameter are removed from the steak and measured with the use of a V-shaped blade, whereas SSF is determined on a single 1 cm thick, by 5 cm wide slice removed from the lateral end of the steak and measured using a flat blade (Shackelford, 1999). Important to note for both measurements, samples are taken parallel to the muscle fibers. Due to known effects of the aging processes on meat tenderness Shackelford et al. (1997) utilized carcasses processed under laboratory (Exp. 1: n = 400, no electrical stimulation) and commercial settings (Exp. 2: n = 554, electrical stimulation) classified by WBSF values (< 6 kg = tender, 6 to 9 kg = intermediate, > 9 kg = tough) to evaluate the ability of WBSF measured at 1 or 2 d postmortem to predict the tenderness of beef aged 14 d. Tender steaks (WBSF < 6 kg) after being aged 14 d were predicted with 84.8 and 94.8% accuracy for Exp. 1 and Exp. 2 respectively. Further assessment by classification reported 100% of the tender steaks would have low WBSF (< 6 kg) at 14 d, however, for intermediate only 81% for Exp. 1 and 85 % for Exp. 2 had low WBSF and in the tough group 74% and 67% of carcasses did not have low WBSF values at 14 d. Shackelford et al. (1999) used longissimus steaks aged 14 d from 479 beef carcasses cooked to internal temperature of 70° C and assessed for SSF and WBSF to predict trained sensory panel tenderness ratings (1 =

extremely tough; 8 = extremely tender). The authors reported a stronger correlation for SSF than WBSF with sensory tenderness rating, with r = -0.82 and -0.77, respectively. As discussed in a previous section, protein turnover is heavily regulated by activity of the calpain system, specifically calpastatin, which inhibits calpain activity and protein degradation, thus affecting WBSF. Lambs expressing the callipyge gene serve as a model to understand the role of calpastatin in protein accretion and the underlying effects on WBSF. Duckett et al. (2000), using longissimus (LD), semimembranosus (SM), supraspinatus (SSM) muscle samples collected from 20 callipyge and 20 non-callipyge lambs harvested at different weights across the growth curve (7, 20, 36, 52, and 69 kg), concluded that calpastatin activity was greater in LM and SM from callipyge lambs compared to controls, which is in agreement with the greater WBSF observed for these muscles compared to non-callipyge lambs. In a study by Koohmaraie et al. (1995), using 40 Dorset wethers harvested at 169 d of age, it was reported that lambs expressing the callipyge gene had 83% greater calpastatin activity than non-callipyge lambs; however, calpain 1 activity was not affected across groups. These authors further noted greater WBSF values in the LD of lambs expressing the callipyge gene at 1, 7 and 21 d postmortem; however, despite sufficient aging (21 d) WBSF values tended to be greater than d 1 non-callipyge values. Thus, a greater amount of calpastatin decreases the amount of protein degradation resulting in the net accretion of protein through muscle hypertrophy, having negative effects on tenderness.

Conclusions of tenderness

Lambs exhibiting the callipyge have greater muscle mass as a result of greater calpastatin activity inhibiting the degradation of protein by calpains resulting in the net accretion of protein. These lambs are also known to have greater WBSF values, thus a less tender product and are

more efficient than lambs that do not express this gene. Recent industry focus has been centered on the improvement of feed efficiency in beef production, as these cattle have the potential to produce more pounds of saleable beef with less inputs. It has been well documented across livestock species that improvements in efficiency may be partially due to differences in protein turnover and this could have negative implications on postmortem muscle degradation Although limited work has been published, cattle selected for improved feed efficiency have been reported to have increased calpastatin activity, suggesting less protein degradation, thus, production of a less tender product. The effects of tenderness and consumer perception of beef have been well documented indicating consumers are willing to pay for products that are guaranteed tender. Tenderness has been, and will continue to be, one of the biggest influencers on consumers willingness to purchase and consume beef products. Therefore, producers need to be reminded of the potential impact selection for improved feed efficiency may have on the quality of beef.

Nutritional Management in Beef production

Roughage versus concentrate: effects on beef quality

A continual debate by consumers is if there is a distinguishable difference between beef from cattle fed roughage or concentrate-base diets. Oltjen et al. (1971) conducted consecutive studies that evaluated roughage and concentrate finishing methods (Trial 1) and pelleted alfalfa: timothy hay combinations (Trial 2) on carcass characteristics and meat quality. In the first trial the authors utilized 48 Hereford steer calves (239 kg) fed either concentrate (A) or pelleted roughage (B) for 77 d and then steers either remained on their respective diet or were assigned to the opposite diet for the last 77 d of the feeding period, which resulted in treatments of AA (continuous concentrate), BB (continuous forage), AB (concentrate to forage), and BA (forage to

concentrate). Carcass grades of steers across treatments were of low (BB, BA) or average (AA, AB) Choice; however, steers fed the high roughage diet had approximately 55% the amount of BF but maintained approximately 80% of ether extract (marbling) in the ribeye. Interestingly, flavor, tenderness, and overall desirability were scored less favorably by sensory panelists for steaks from AA and AB treatments compared to BB and BA treatments. This difference is of particular interest as it contradicts present paradigms that beef from steers finished on high-concentrate diets is more desirable and more tender. Because the roughage fed steers had less subcutaneous fat, there is potential that lack of fat cover could increase carcass exposure to the negative effects of cold shortening, which would ultimately result in a tougher product; however, that effect was not noted in this trial. In a second study using 48 Hereford steer calves (254 kg) Oltjen et al. (1971) evaluated pelleted combinations of alfalfa: timothy hay in all forage diets, with treatments designated as: B (98.5 alfalfa: 0 timothy), C (67.6 alfalfa: 30.9 timothy), D (34.3 alfalfa: 60.7 timothy, 2.5 molasses, 1.0 urea), and E (0 alfalfa: 91.5 timothy, 5.0 molasses, 2.0 urea). Steers were harvested on 203, 210, 217 and 224 d for diets B, C, D, and E, respectively, with all steers receiving the USDA Select quality grade, regardless of treatment. The authors noted steaks from steers fed diet B were rated most tender compared to all other treatments, with no differences noted for other palatability traits (juiciness, overall desirability). In a comparison of carcasses of identical USDA Select quality grades (n = 30) produced from forage or grain finishing systems, Bowling et al. (1977) concluded that grain finished steers had greater amounts of subcutaneous fat and longer sarcomeres which correlated to the lesser WBSF values measured on LD steaks from grain finished steers. In contrast, carcasses from forage finished steers had lesser amounts of subcutaneous fat, shorter sarcomere length and consequently greater WBSF values (Bowling et al., 1977). The differences noted in WBSF values by Bowling et al. (1977) of

steaks from roughage vs. concentrate fed steers may have been a result of two contributing factors: greater amounts of connective tissue in forage finished cattle, as well as changes in sarcomere length during chilling. Carcasses of forage finished steers experienced approximately 28.4% shortening of the sarcomeres compared to 17.2% observed in grain finished cattle, which is less than the threshold to determine differences in tenderness at approximately 20% shortening (Bowling et al., 1977).

Evaluating the effect of high forage vs. concentrate feeding on steaks produced from steers finished to a similar carcass composition, Young and Kauffman (1978) utilized 42 yearling Hereford steers fed one of three diets: 1) a grain diet (66% cracked corn, 27.9% corn silage, 4.5% SBM, DM basis), 2) corn silage (92.2% corn silage, 7.1% SBM, DM basis), 3) or 50% haylage: 50% corn silage diet (53.2% corn silage, 43.1 haylage, 2.9% SBM, DM basis). Evaluation of tenderness, juiciness, and flavor were conducted by an experienced sensory panel on rib steaks and rib roasts aged 7 days. The authors concluded steaks and roasts produced from corn silage and the haylage-corn silage mix were rated equal to or better than samples from grain fed steers. Bowling et al. (1978), using 100 Santa Gertrudis steer calves harvested either as calves, yearlings, long-yearling or two year olds, evaluated the effects of all forage (grass), grain on grass, or drylot feeding management systems on beef quality characteristics. Trained sensory panel analysis reported flavor and juiciness ratings were more desirable for steaks from the LD for steers finished on grain, while steaks from long-yearlings finished on grass or supplemented with grain on grass were less tender than steaks from all other management systems. The authors further identified an association between BF and tenderness as well as BF and shear force, suggesting that as BF is increased from 1 to 7 mm sensory tenderness ratings increase ($r^2 = 0.19$) and decreases shear force ($r^2 = 0.29$; Bowling et al., 1978). The responses of increased BF are

typical of cattle finished on high-grain diets for longer periods of time as the latter portion of the growth curve is characterized by greater deposition of fat, with minimal or no protein accretion. Bidner et al. (1981), used 46 Hereford × Angus steers fed one of four dietary treatments (A: bermudagrass/ ryegrass pasture, free choice hay, with 0.9 kg of cottonseed meal/ steer daily when forage was limiting; B: grain supplemented at 1% of BW in addition to ryegrass and bermudagrass; C: ryegrass pasture, followed by 60 days of 1% of BW grain and bermuda grass pasture, finished for 70 days on concentrate diet; D: ryegrass and bermudagrass pasture followed by 74 days of concentrate diet). All steers were fed to a similar final BW (476 kg) with USDA quality grades of low Select (A), high Select (B), and low Choice (C,D). Evaluation of WBSF was determined from loin steaks and sensory analysis was conducted by a 168 – household consumer panel on two chuck, two loin, and two round steaks, from which the authors observed no differences in shear force or organoleptic properties (tenderness, juiciness, flavor, overall desirability) across treatments. A potential factor leading to the lack of differences noted in the study by Bidner et al. (1981) may have been differences in sensory analysis as previous studies utilized trained sensory analysis where steaks are typically cooked to a similar degree of doneness. In contrast, the household assessment used in this study (Bidner et al., 1981), did not provide cooking instructions, thus the degree of doneness was determined by consumers preference, potentially affecting the organoleptic properties resulting in no differences across treatments. Similar results were documented by Duarte et al. (2013) using Nellore (n = 17) and Angus (n = 17) steers 20 months of age fed different concentrate: roughage ratios, either 100:0 or 70:30 for 84 d concluding there were no differences for calpastatin activity, myofibril fragmentation index and WBSF of LD steaks between dietary treatments. Moloney et al. (2007) conducted a pair of experiments using 28 Charolais × Friesian steers 19 months of age (Exp. 1),

and 36 Charolais × Friesian and 18 Friesian steers (Exp. 2), to evaluate different feeding regimens on beef quality. In Exp. 1 steers were offered diets consisting of either grass silage *ad libitum* plus 6.35 kg of concentrates or grass silage as the sole feed *ad libitum* for a period of 35 d followed by *ad libitum* concentrate until harvest. Treatments used in Exp. 2 consisted of *ad libitum* access to grass silage plus 6 kg of concentrate (control), *ad libitum* concentrate (CD0), or grass silage for 112 d followed by *ad libitum* concentrate until harvest (CD112). At harvest LD samples were collected and aged for 2, 7, 14 d for sensory and WBSF measurements. The authors reported no difference in tenderness as measured by WBSF values or sensory panel across all aging treatments in Exp. 1. In Exp. 2 WBSF was greater for CD0, and CD112, compared to control steers; however, after 7 or 14 d of aging WBSF values were only greater in LD samples from the CD0 treatment, suggesting with proper aging concerns of meat tenderness are minimal. Review of the literature supports the underlying theme that differences in meat tenderness from roughage finished vs. concentrate finished beef are responses to differences in altered carcass composition, particularly BF, potentially exposing carcasses to faster rates of chilling causing sarcomeres to be shortened; subsequently decreasing meat tenderness by increasing the force required to shear the muscle fibers.

Historically, consumers have been enticed to consume beef due to perceived advantages in the tenderness, juiciness, and flavor of concentrate fed beef; however, in the past decades consumers have challenged the quality of beef produced from concentrate versus roughage feeding systems. Evidenced by work reported previously, consumers have a preference for grain finished beef for the organoleptic properties of juiciness and flavor. Interestingly though, consumers are able to discern differences in tenderness, the number one indicator of beef quality. Differences in tenderness amongst concentrate and roughage fed steers for both sensory analysis

and WBSF measurements are inconsistent, but the general trend supports a more tender product from concentrate vs. roughage fed cattle. It has been well understood that cattle finished on roughage based systems have a lesser degree of subcutaneous fat, therefore the disparities noted in tenderness amongst roughage and concentrate fed beef may be attributed to the effects of cold shortening of the muscle sarcomeres during processing at harvest, thus, decreasing tenderness. Diets containing large quantities of roughage have the potential to impact tenderness; however, when managed properly within cattle feeding systems provide advantages in cattle performance.

Value of roughage in beef cattle diets

One particular advantage of ruminants is their ability to utilize fibrous feeds to support production; however, to optimize performance of cattle across the feeding period different nutritional management strategies are implemented. In the most recent U.S. feedlot consultant survey conducted by Samuelson et al. (2016), nutritionists' reported including 30% or greater roughage on DM basis in receiving diets for lightweight and yearling calves. Of those responding to the survey, 58.3, 8.33, 4.17, and 4.17% of nutritionists reported using alfalfa hay, cottonseed hulls, corn silage, and corn stalks as the primary roughage source, respectively. During transition to finishing diets 56.3% of nutritionists' reported using a multiple step-up diet approach with approximately 40.7% roughage in the initial diet and decreasing roughage inclusion using 4 different diets within a 24 d period to achieve approximately 8-10% roughage in the final diet. The alternative choice used by 40.6% of nutritionists was the 2-ration blending method containing a reported average of 38.8% roughage. The authors further report inclusions of roughage in finishing diets are typically 8 to 10% on a DM basis, which is accomplished primarily through the use of corn silage (37.5% of respondents) and corn stalks (29.2% of

respondents). Alternative sources of roughage differ across geographical locations but the most common include: sorghum silage, sudan grass hay, and wheat straw.

In contrast to the U.S. beef industry, Millen et al. (2009) conducted a survey of 31 nutrition consultants evaluating the nutritional management practices in the Brazilian beef industry, where cattle typically are of *Bos Indicus* origin. Similar strategies to those used in the U.S. for receiving and transitioning cattle are used in Brazil through the use of multiple step-up diets (50% of programs); however, this is accomplished on average using 3 diets in a period of 17 days, with the initial transition diets containing approximately 55% roughage. The second most common (19%) feeding regimen used to transition cattle is to limit feed the final diet, with incremental increases to bring cattle up on full feed slowly. From the survey, the authors reported that 52% of nutritionists formulate finishing diets to contain between 51 to 65% grain, where as 23% use 36 to 50% grain, 19% report 20 to 35% grain, and the remaining 6% reported between 66 and 80% corn; however, grain concentration did not surpass 81%. Alternatively, Brazilian nutritionists reported using greater amounts of roughage in finishing diets than are commonly used in the U.S., reporting on average 29% inclusion of roughage, with values ranging from 12% to 45%. The authors speculate such low inclusions of grain are a result of economics in the market as carcasses are not awarded for greater degrees of marbling as they are in the United States beef industry.

Across nutrition consultant surveys from the U.S. and Brazil, the use of step-up diets has been widely adopted practice as cattle are transitioned to high concentrate diets. The primary purpose of utilizing this management strategy is in the prevention of metabolic disease, particularly acidosis. Acidotic conditions in cattle result from the rapid intake of highly fermentable carbohydrates altering the pH of the rumen, which results in a decrease of rumen

function while subsequently altering performance in cattle (Owens et al., 1998). Therefore, to mitigate the negative effects of acidosis Owens et al. (1998) suggests feeding greater amounts of dietary roughage, decreasing the amount of processing of concentrate sources, or the incorporation of a limit feeding strategy. Ruminants require a minimum amount of forage in order to maintain rumen function and health which aids to maximize performance; however, it has been widely debated amongst ruminant nutritionists how to best utilize NDF analysis from various feedstuffs to assign roughage values that can assist in the formulation of diets to ensure proper rumen health.

Defoor et al. (2002) evaluated the effects of roughage source and concentration on performance and DMI of beef heifers. In the initial experiment Defoor (2002) used 12 beef heifers (389 kg) in three simultaneous 4 × 4 Latin square trials fed either alfalfa hay, sudan hay, wheat straw, or cottonseed hulls at 5, 10, or 15% of diet DM, concluding that NE_g intake/kg $BW^{0.75}$ increased as NDF from roughage increased with all treatments tending to have greater NE_g intake/kg of $BW^{0.75}$ than alfalfa hay. The authors suggested roughage sources with greater NDF concentrations have greater value than roughages which contain lesser concentrations of NDF (Defoor et al., 2002). Therefore, forages with greater NDF value can be utilized in diets at a lesser concentration than those with lesser NDF concentrations. In a follow up experiment using 105 beef heifers (275 kg), the authors evaluated the methods by which to exchange roughage in a 140-d finishing trial (Defoor et al., 2002). Alfalfa hay included at 12.5% of the diet was used as the control, and was compared to cottonseed hulls and sudan silage at different concentrations: exchanged equally with alfalfa hay on DM basis (12.5% cottonseed hulls, 12.5% sudan silage), an equal NDF basis (5.9 % cottonseed hulls, 7.1 % sudan silage), or on retained NDF basis (2.5% cottonseed hulls, 3.2% sudan silage), defined as particles greater than 2.36 mm

contributing to NDF. The authors concluded that as concentration of cottonseed hulls increased from 2.5% to 12.5% there was a linear increase in ADG, DMI, G:F, and NE_g intake/kg of $BW^{0.75}$, whereas for sudan silage a quadratic effect for DMI was observed with the greatest intake at 7.1% inclusion, with a similar trend observed for ADG and NE_g intake/ kg $BW^{0.75}$. A meta-analysis conducted by Galyean and Defoor (2003) of 11 trials showed a close relationship between DMI and dietary NDF coming from roughage ($r^2 = 0.92$), from which the authors suggest when exchanging roughage sources in feedlot diets it may be more practical to do so by factoring in the amount of NDF contributed by the individual roughage source. These data suggest roughage sources with greater concentrations of NDF are of greater value in high concentrate finishing diets, and can be used in lesser dietary concentrations to elicit similar responses in cattle performance while maintaining rumen health.

Role of roughages in dilution of energy and feedlot performance

As illustrated in the previous section a minimum inclusion of roughage in finishing diets is important for the maintenance of rumen health parameters in order to optimize performance. A limitation of using roughages in high concentrate diets is the concern for energy dilution, which Galyean and Defoor (2003) define as the subsequent increase in DMI by cattle to maintain energy intake as roughage displaces grain in high concentrate diets. This is further demonstrated using values from the NRC (2016) which establishes a NE_g value for dry-rolled corn of 1.49 Mcal/kg of DM (TDN = 87.6% DM), however, common roughages such as corn silage or alfalfa hay have NE_g values of 0.96 (TDN = 67.7% DM) and 0.59 Mcal/ kg of DM (TDN = 55.2% DM), respectively, meaning the energy value of the diet is decreased when corn is replaced with roughage. The energy value of roughages is also lessened when fed in conjunction with high

grain diets, as the lesser ruminal pH creates an unfavorable environment for fiber digestion. This concept is referred to as negative associative effects.

Evaluations of dietary energy dilution across various levels of forage inclusion have been studied extensively. Stock et al. (1990), utilizing 216 British-Continental crossbred steers evaluated the addition of a roughage blend, composed of 50% alfalfa silage: 50% corn silage at 0, 3, 6, or 9% of diet DM on performance of steers receiving diets containing high-moisture corn and dry-rolled sorghum grain. The authors noted that as roughage inclusion increased, DMI increased (10.31, 11.36, 11.35, 11.46 kg/d) and G:F decreased linearly (0.157, 0.146, 0.148, 0.138) for 0, 3, 6, and 9% roughage inclusion, respectively. Steers fed the dry-rolled sorghum grain had greater intake of concentrate but were less efficient, suggesting the observed differences in FE were not solely due to energy dilution but were also impacted by the digestibility of the grains. Similarly, Stock et al. (1990) found G:F of British-Continental crossbred steers (n = 648) was decreased as inclusion of a corn silage: alfalfa haylage blend was increased (0, 3.75, 7%) in DRC diets vs. 75% DRC: 25% dry rolled wheat diets. Guthrie et al. (1996) evaluated the effect of roughage source and dietary inclusion (Exp 1), roughage source and grain processing (Exp. 2), and roughage value of alfalfa hay vs. sudan grass hay at different dietary concentrations (Exp. 3). The initial study (Exp 1) utilized 282 British × Brahman heifers in a 2 × 3 factorial design with either 7.5 or 15% dietary inclusion of alfalfa, sorghum-sudan grass hay, or cottonseed hulls, from which the authors concluded ADG was not different between roughage inclusions, although heifers that received 15% roughage consumed 7% more DMI/d than heifers on the 7.5% roughage diet, thus, efficiency was poorer in heifers receiving the 15% roughage diet. In Exp. 2, Guthrie et al. (1996), using 224 British × Continental crossbred steers in a 2 × 2 factorial, fed 90% concentrate diets consisting of either whole shell corn or steam

flaked corn with approximately 10% inclusion of alfalfa hay or sorghum-sudan grass hay. The authors reported no interaction between grain processing and roughage inclusion; however, despite no differences in ADG, DMI of steers receiving sorghum-sudan hay was greater than alfalfa hay fed steer. The authors attributed the increase in DMI to an alteration in rumen kinetics in response to the lesser quality of the sorghum-sudan hay compared to alfalfa hay, as noted by increased ADF content of the sorghum-sudan hay (Guthrie et al., 1996). Greater feed conversion (F:G), or pounds of feed per pound of gain, as observed by Guthrie et al. (1996) is undesirable in the beef industry as it is inversely related to feed efficiency, meaning as feed conversion increases, feed efficiency is poorer. In Exp. 3, Guthrie et al. (1996) utilized 132 British × Continental crossbred steers fed high concentrate diets (approximately 90% concentrate) with either 5, 7.5, or 10% sorghum-sudan hay, with a 10% alfalfa hay diet used as a negative control, noting a greater DMI for steers receiving the sorghum-sudan hay compared to control steers. However, across the different levels of sorghum-sudan hay inclusions, a quadratic effect indicated greater DMI and ADG by steers fed 7.5% sorghum-sudan hay. No differences were noted in feed conversion across roughage sources; however, with sorghum-sudan hay treatments as dietary inclusion increased feed conversion tended to increase, thus, steers were less efficient. In the latter two studies by Guthrie et al. (1996), differences in DMI may have been a result of the greater ADF content of the sorghum-sudan hay, which indicates that the fiber was less digestible and lower in energy potentially causing an increase in DMI. In a series of trials, Loerch and Fluharty (1998) evaluated the effects of concentrations of roughage and concentrate processing on steer performance. Trial 1 utilized 108 crossbred steers (295 kg) and offered either: 1) 85% concentrate diet for 186 d, 2) 100% concentrate diets (186 d, whole-shelled high moisture corn), 3) 85% concentrate for 84 d followed by 100% concentrate for 102 d, or 4) 100%

concentrate for 84 d followed by 85% concentrate for 102 d, with the remainder of the diet (approximately 15% DM) consisting of corn silage, the primary roughage source. The authors concluded that steers switched to the 85% concentrate diet during the latter portion of the feeding period had greater DMI than steers fed 100% concentrate diet with no additional gain; however, during the finishing period feed efficiency was greatest for 100% concentrate and least for steers fed 85% concentrate for the duration of the trial with no resulting effects on carcass characteristics. In a subsequent study, using 108 crossbred steers in a 158 d finishing trial the authors evaluated the effects of roughage levels and corn processing (whole vs. rolled high moisture corn) on steer performance by altering inclusions of concentrates on d 56 and 112 by either increasing (70, 85, 100%) or decreasing (100, 85, 70%) concentrate levels, with corn silage included as the roughage source. The authors noted during the first 56 d period steers which received the 70% concentrate consumed approximately 19% more feed and grew 1% faster than steers receiving 100% concentrate diets. During the final period (d 113 to 158) ADG was not affected by concentrate levels or corn processing; however, there was a tendency for steers fed rolled high moisture corn at 100% dietary inclusion to have lesser ADG (approximately 14%) than all other dietary treatments. The depression in ADG is likely due to the fact that starch from processed grains is more readily fermented in the rumen, potentially increasing the risk of acidosis in these cattle, thus decreasing performance. Bartle et al. (1994) evaluated the effects of roughage level (10, 20, 30% roughage equivalent; RE), roughage source (alfalfa hay vs. cottonseed hulls) and feeding regimen (constant RE vs 2% RE during mid-finishing period) using British crossbred and *Bos indicus* crossbred steers fed steam flaked sorghum grain diets for 124 to 166 d (n = 432, BW = 326 kg), concluding as RE increased DMI increased and ADG was decreased, due to the dilution of dietary energy with increases in

roughage. A similar trend was observed when steers were fed cottonseed hulls compared to alfalfa hay. Differences may be attributed to differing particle size of the roughage sources altering rumen kinetics. The smaller particle size of the cottonseed hulls may potentially lead to an increase in the rate of passage, decreasing digestibility, may result in a decrease in ADG despite an increase in DMI. Interestingly though, decreasing roughage to 2% RE during mid-finishing improved overall G:F by 2, 7 and 24 % for steers on diets that contained 10, 20, 30% RE, respectively. The authors suggest that the improvement in overall G:F is due to an increase in available dietary energy when RE was reduced to 2%, which elicited a compensatory gain response by steers with that effect being greater in steers that were previously fed diets with greater RE. Kriekemeier et al. (1990) using 126 crossbred steers (334 kg) offered a steam-rolled wheat diet without roughage or a mix of 50% alfalfa hay: 50% corn silage at 5, 10, or 15% of diet DM, reported as dietary roughage increased there was a linear increase in DMI. However, a quadratic effect was noted for ADG, F:G, and HCW in that steers fed 5 or 10% roughage gained faster, had lesser feed conversion ratios and railed heavier carcasses than control or 15% roughage fed steers. It has been well established that higher inclusions of roughage in finishing feedlot diets decreases the dietary energy concentration, and can subsequently decrease efficiency. Further research is needed to evaluate the relationship between forage quality and levels of dietary inclusion, which may result in the optimization of steer performance.

Conclusions on roughage

Evaluations of roughage inclusions, corn processing, and energy dilution effects on performance of ruminants has been extensively researched. In support of the concept of energy dilution, increases in dietary roughage concentration cause an associated increase in DMI with a

concomitant decrease in G:F, which subsequently may impact steer performance. Typical roughage inclusions in finishing diets in the U.S range between 8-10% (Samuelson et. al., 2016). Although there is potential to further improve steer FE at even lesser roughage levels, approaching 5-8% roughage, additional management may be required in order to avoid acidosis and to successfully feed finishing diets containing low levels of roughage. Roughage inclusion rates are dependent on physical and metabolic characteristics of the grain used (whole, rolled/cracked, steam-flaked) in combination with forage quality. A gap in our knowledge exists on the ability to effectively assign a value to roughage sources and our ability to efficiently utilize them in order to optimize performance. Analysis of fiber most commonly utilizes NDF and ADF, extending further into effective NDF (eNDF). The use of eNDF or the amount of NDF coming from a particular roughage may provide opportunity to utilize fibrous feeds which more effectively stimulate rumination at lesser inclusions in diets, while maintaining rumen health and improving economic efficiency. Evaluation of individual roughages will be critical in the future, particularly as cellulosic extraction in support of biofuel production and other processing methods are developed changing the nutritional profile of roughages, altering their use in diets. The effects of roughages on rumen health in combination with ruminants ability to utilize low quality roughages for the production of lean is advantageous to the sustainability of the beef industry.

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CHAPTER 3.

INFLUENCE OF FEED EFFICIENCY ON MEAT TENDERNESS ATTRIBUTES OF BEEF STEERS

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Abstract

Enhancing feed efficiency (**FE**) in beef cattle is at the forefront of the beef industry. Previous work suggests improving FE may alter calpain system activity, potentially affecting meat tenderness; therefore, the study objective was to assess effects of beef cattle FE on postmortem meat tenderness. Crossbred steers were grown at the University of Missouri (76 d) on a whole-shell corn (**MU-Corn**, *n* = 90) or roughage-based diet (**MU-Rough**, *n* = 91), phenotypically classified for FE based on residual feed intake (**RFI**) calculations and finished at Iowa State University (**ISU**). Within each growing phase (**GP**) diet, the 12 greatest (**HFE**; average RFI -3.33 ± .77, SD) and 12 least (**LFE**; average RFI 2.90 ± .94) feed efficient steers (48 steers total) were assigned to pens with GrowSafe bunks and transitioned to corn (**ISU-Corn**) or byproduct-based diets (**ISU-Byp**; 87 d) for the finishing phase **(FP).** Rib sections were collected from the 48 steers, and aged for 2 or 14-days for further analysis of calpastatin (d 2), troponin-T (d2, d14), and Warner-Bratzler Shear Force (**WBSF**; d 14). Data were analyzed using PROC MIXED of SAS with fixed effects of MU diet, ISU diet, FE classification, and the interactions;

significance was determined at $P \leq 0.05$. Dressing percent and ribeye area (**REA**) were greater in LFE steers than HFE steers ($P \leq 0.04$), and REA was increased in ISU-Corn vs. ISU-Byp steers ($P = 0.01$). Steak lipid content tended ($P = 0.08$) and marbling score was ($P = 0.03$) affected by ISU diet × FE, with greater lipid in steaks from LFE steers vs. HFE steers within ISU-Byp, with no differences due to FE within ISU-Corn. No interaction between diets and FE classification ($P \geq 0.19$) were observed for WBSF, calpastatin activity, d 2 or d 14 troponin-T degradation. However, MU-Rough steers had greater WBSF than MU-Corn steers ($P = 0.05$). Day 2 calpastatin activity tended ($P = 0.10$) to be greater in HFE steers than LFE steers. No differences were observed in d 2 troponin-T degradation ($P \geq 0.12$); however, d 14 troponin-T was greater in ISU-Corn vs. ISU-Byp steers ($P = 0.005$). Influence of FP feed efficiency (G:F) was determined fitting a slope regression model. Slope estimation for d 2 calpastatin ($P = 0.002$) and d 2 troponin-T ($P = 0.04$) differed from zero only for LFE steers receiving MU-Corn:ISU-Byp.; while the d 14 troponin-T ($P = 0.04$) slope for HFE steers receiving MU-Rough:ISU-Corn differed from zero. These data suggest that high-fiber diets may have a greater impact on meat tenderness than improvements in cattle FE and further work is needed to clarify the role of finishing period FE on meat tenderness attributes.

Key words: cattle, feed efficiency, meat tenderness

Introduction

Sociological and economic influences are driving the beef industry to make marked improvements in feed efficiency (**FE**; Basarab et al., 2003). Richardson and Herd (2004) suggested that differences in protein metabolism, tissue metabolism and stress may explain up to

37% of the variation in feed efficiency differences among cattle. Components of the Ca^{2+}-dependent proteolytic calpain system critically regulate protein metabolism, especially calpastatin. Calpastatin inhibits the degradation of proteins by calpain 1 in the presence of Ca^{2+}, thus, greater calpastatin activity results in accretion of protein. For example in callipyge lambs, increased muscle development is contributed in part to increased calpastatin activity; however, Warner-Bratzler Shear Force (**WBSF**) of the loin is also increased in these animals, compared to non-callipyge lambs (Duckett et al., 2004). Thus, increased calpastatin activity in relation to calpain activity is indicative of decreased protein degradation, resulting in a less tender steak.

Beef is a highly preferred protein source, and consumer perceptions of beef quality are driven in part by tenderness (Miller et al., 2001, Corbin et al., 2014). Improvements in FE must be made with the end consumer in mind; however, limited work in beef cattle has examined the implications of FE on meat tenderness. In the work of McDonagh et al. (2001) a single generation selection for improved RFI resulted in a 13% increase in calpastatin activity in steaks from highly efficient steers. Increased calpastatin activity may contribute to increased efficiency, as less protein degradation should increase net accretion of muscle. Unfortunately, decreased protein degradation post mortem results in a less tender, less desirable beef product. Therefore, the objective of this study was to evaluate meat tenderness attributes of steaks from steers identified as phenotypic extremes for FE.

Materials and methods

Experiment 1

Experimental design & sample collection.

All protocols and procedures of this trial involving live animals were approved by the University of Missouri and Iowa State University animal care and use committees.

One hundred and eighty-one British × Continental crossbred steers of sale barn origin were transported to the University of Missouri for the growing period. Steers were randomly assigned to pens equipped with GrowSafe bunks, receiving either a roughage-based diet (**MU-Rough,** *n* = 91) or whole shell corn-based (**MU-Corn,** *n* = 90) diet for 76 d (**Table 1**). All steers were RFI phenotyped according to the methods of Basarab et al. (2003) at the end of the growing period and then transported to the Iowa State University Beef Nutrition Farm (Ames, IA). Forty-eight steers were then selected for further study, including the twelve greatest (highly FE, **HFE,** negative RFI) and least efficient (lowly FE, **LFE,** positive RFI) steers from their respective growing phase diets. Within growing phase diet type steers were assigned equally to a byproduct-based finishing diet (**ISU-Byp**) or a cracked corn-based finishing diet (**ISU-Corn;** Table 2) for 87 d (*n* = 6 per treatment combination). Further details about the live animal portion of this study have been previously reported by Russell et al. (2016b). Summary statistics for growth and FE parameters for the 48 steers during the growing phase are shown in Table 3.

Steers were harvested on d 191 at a commercial abattoir (Tyson Fresh Meats, Denison, IA) and HCW collected. Carcass data collected after a 24 h chill included: ribeye area (**REA**), percent kidney, pelvic, heart fat (**KPH**), back fat (**BF**), marbling scores (**MS**) and yield grades (**YG**) according to USDA standards. Dressing percent (**DP**) was calculated as

$$(\text{HCW} \div \text{final BW}) \times 100$$

using an average final BW from consecutive weight dates with a 4% pencil shrink applied. Rib sections, approximately 6.35-cm thick, were removed from the right side of each carcass at the 12th rib between 21-24 hours post-exsanguination, and transported on ice to the Iowa State University Meat Laboratory for further processing. One, 2.54-cm thick steak was removed from each section, vacuum packed and aged 14 d in a standard display cooler (7.0 lux of Deluxe Cool White fluorescent light; constant lighting 24 h/day; Osram Sylvania, Danveres, MS) at 2° C for analysis of WBSF**,** pH, Minolta objective color scores (L*, a*, b*), and percent cook loss. Remaining sample was minced, mixed for homogeneity, divided into two approximately 10 g samples and packaged. One sample was stored at -20°C for d 2 analyses, with the second being vacuum packaged, aged for 14 d in a standard cooler at 2° C, and then frozen at -20° C for d 14 analyses. Steaks were analyzed for calpastatin activity (d 2), moisture, fat and protein (d 2), calpain 1 autolysis (d 2), and troponin-T degradation (d 2, 14).

Warner-Bratzler shear force, pH, color scores, percent cook loss

In preparation for analysis of WBSF, pH, color and percent cook loss, steaks were thawed at 2° C for 48 h. Prior to cooking steaks pH was measured using a HI 9025 microcomputer pH meter attached to a Hanna FC 200 probe (Hanna Instruments, Inc. Ann Arbor, MI), followed by measurements of Minolta objective color scores (L*, a*, b*) using a Minolta CR-310 colorimeter (Konica Minolta, Inc. Omaha, NE; D65 light source, 0^{O} viewing angle, 50 mm aperture). Steaks were weighed prior to and post cooking to allow for the calculation of percent cook loss. The mechanical measurement of tenderness was determined on all steaks using WBSF. Sample preparation and measurements were conducted according to modified methods reported by Pogge et al. (2014a), modifications included steaks being cooked

to an internal temperature of 69° C ± 1°, and removal of four cores per steak for analysis. Data from the four cores were averaged to generate a steak mean.

Proximate analysis

Percent moisture, protein, and fat were determined from a sub sample prepared for analysis by homogenizing the sample in a food processor (model Ninja Chopper, Newton, MA). Percent moisture was determined according to the Association of Official Analytical Chemists (2006a, chapter 39). Protein concentration was measured in duplicate on 1 g of sample using a Leco N analyzer (model TruMac N, St. Joseph, MO). Analysis of crude fat was conducted using a modified procedure of the FOSS Soxtec system (FOSS North America, Eden Prairie, MN). Approximately 2 g of sample was hydrolyzed in 4N hydrochloric acid for 1 h using a Soxcap 2047 unit, then transferred to a microwave oven at 30% power for drying in intervals (40, 30, 30, 30 min) with a 5 min rest between intervals for a total of 130 min. Aluminum beakers containing 5 glass beads were weighed prior to insertion of samples and beakers into the Soxtec 2055 system for extraction using n-hexane. After extraction beakers were placed in an oven at 100˚C for a minimum of 45 min, transferred to a desiccator to cool for 45 min, and then weighed for the determination of fat content.

Troponin-T, calpain 1 autolysis

Extraction of whole muscle protein was conducted according to the methods of Lonergan et al. (2001). Methods previously reported by Bechtel and Parrish (1983) were used for the determination of the protein concentration using a Lowry assay kit (BioRad protein assay kit, BioRad Laboratories, Hercules, CA) and preparation of samples for Western blot analysis. Gel

composition and Western blotting were conducted according to modified methods previously reported by Rowe et al. (2004) for the analysis of calpain 1 and troponin-T. Procedural modifications are related to differences in antibody dilutions and the use of an 8% acrylamide gel for calpain 1 analysis. Primary antibody used for measurement of troponin-T was monoclonal anti-troponin-T (T6277: Sigma-Aldrich, St. Louis, MO) diluted at 1:20,000. For calpain 1 determination the primary antibody used was monoclonal anti-calpain 1 (MA3-940: Affinity Bioreagents, Inc., Golden, CO) diluted at 1:5,000. A similar secondary antibody, anti-mouse IgG peroxidase conjugate (A-2554; Sigma Aldrich) diluted at 1:10,000, was used for analysis of both calpain 1 and troponin-T. Immunodetection of proteins was determined using the Amersham ECL Prime Western Blotting Detection Reagents (GE Healthcare UK Limited, Buckinghamshire, UK).

Calpastatin activity

A fresh, refrigerated d 2 muscle sample (10 g) was used for the isolation of calpastatin according to the heated calpastatin preparation procedure previously reported by Shackelford et al. (1990). Prepared samples were then used for the determination of calpastatin activity using casein as a substrate according to the methods of Shackelford et al. (1990). Activity of calpastatin was further calculated from individual sample weight and protein concentration and values are reported as calpastatin activity per gram of protein.

Statistical analysis

Data were analyzed using the MIXED procedure of SAS version 9.4 (SAS Inst. Inc., Cary NC) as a 2×2×2 factorial with steer as the experimental unit (n = 6 per treatment

combination). The model included the fixed the effects of MU diet (MU-Rough and MU-Corn), ISU diet (ISU-Byp and ISU-Corn), FE classification (HFE or LFE), and the interactions. Data reported are LSmeans and standard error of the mean (SEM). Analysis for outliers was conducted using the Cook's D procedure; an outlier was determined at a Cook's D value > 0.5. Significance was determined at $P \leq 0.05$, and tendencies from $0.05 < P \leq 0.10$.

Experiment 2:

Methods and statistical analysis

To assess the effects of finishing phase feed efficiency on postmortem tenderness attributes of beef, a reanalysis of the data was conducted. For this analysis G:F, an alternate continuous variable measurement of feed efficiency, was used. A slope regression model was fit to evaluate the effect of FP feed efficiency (G:F) on protein expression, with each growing x finishing diet combination evaluated within GP FE classification. Diet combinations were assigned numerically in SAS as follows: diet 1: MU-Corn: ISU-Byp; diet 2: MU-Corn: ISU-Corn; diet 3: MU-Rough: ISU-Byp; diet 4: MU-Rough: ISU-Corn. Significance was determined by thresholds listed for Exp.1. A significant *p*-value indicates that the slope of the line is different from zero.

Results

Experiment 1: Effect of growing phase FE classification

Carcass characteristics

Carcass data are presented in **Table 4**. The interaction between MU diet, ISU diet, and FE was not significant for any carcass variable ($P \geq 0.41$). There were no two-way interactions

for HCW, KPH, or REA ($P \geq 0.11$). No differences were noted due to MU diet, ISU diet or FE classification ($P \geq 0.17$) for HCW and KPH. The interaction of MU diet and ISU diet ($P = 0.01$) on DP indicates that steers grown on MU-Corn and finished on ISU-Byp had a greater DP than all other diet combinations. Interestingly, FE classification affected DP ($P = 0.012$), with LFE steers having greater DP than HFE steers. Similarly, steers classified as LFE had larger REA than their HFE counterparts ($P = 0.05$; **Figure 1**). There was an effect of FP diet on REA ($P = 0.01$) with steers receiving the ISU-Byp diet producing carcasses with larger REA than those finished on corn. A similar interaction between MU diet and ISU diet was noted for BF thickness ($P = 0.003$) and USDA YG ($P = 0.001$), with steers grown and finished on corn having the greatest amount of BF corresponding to the greatest YG, and steers grown on roughage and finished on corn having least amount of BF combined with the least YG. A second interaction in YG data was identified between ISU diet and FE classification ($P = 0.05$), with HFE steers receiving the ISU-Corn diet having the greatest YG compared to all other combinations. Marbling scores presented in **Figure 2A** illustrate an interaction between ISU diet and FE classification ($P = 0.04$), in which LFE steers finished on ISU-Byp had greater marbling scores than all other treatments.

Minolta color scores, percent cook loss, proximate analysis, protein expression, WBSF, calpastatin activity

Minolta objective color scores, percent cook loss, proximate analysis, protein expression, WBSF, and calpastatin data are presented in **Table 5** and **Figure 3**. The two and three-way interactions between MU diet, ISU diet and FE were not significant for any variable ($P \geq 0.12$), with the exception of fat content ($P \geq 0.08$). No differences in Minolta objective color scores

(L*, a*, b*), percent cook loss, moisture, or protein content of steaks due to treatments were noted ($P \geq 0.16$). However, a tendency for a difference in fat content of steaks ($P = 0.08$) reveals that LFE steers fed ISU-Byp diet produced steaks with a greater fat content than HFE steers; however, no difference was noted among steers finished on corn (**Figure 2B**). Calpastatin activity, measured 2-d post mortem showed no difference due to growing or finishing diet type ($P \geq 0.18$). However, a tendency was observed due to FE classification, with steaks from HFE steers having greater ($P = 0.10$) activity of calpastatin than steaks from LFE steers. There was no difference in calpain 1 autolysis or troponin-T degradation ($P \geq 0.15$) due to diet type or FE classification, measured 2-d post mortem. However, d14 troponin-T degradation indicates that steaks from steers finished on ISU-Corn diet had a greater ($P = 0.005$) extent of degradation than steaks from ISU-Byp finished steers ($P = 0.005$) despite no differences observed in d 2 troponin-T. No difference was observed in d 14 troponin-T due to MU diet ($P = 0.12$) or FE classification ($P = 0.13$). Measures of WBSF on steaks aged 14 d indicate no difference in shear force due to ISU diet type ($P = 0.24$) or FE classification ($P = 0.74$); interestingly though, MU diet had an effect on WBSF with steers grown on MU-Rough diet producing steaks that had a greater (P = 0.05) WBSF than steaks from steers grown on MU-Corn.

Experiment 2: Effect of finishing phase feed efficiency on markers of beef tenderness

Calpain 1, protein calpastatin, troponin-T degradation, and WBSF slope estimation data are presented in **Figures 4 - 8**, respectively. Slope estimations did not differ from zero for any diet combination for calpain 1 or WBSF ($P \geq 0.11$). Additionally, slope estimates for calpastatin activity per gram of protein and d 2 troponin-T degradation were not different from zero ($P \geq 0.12$) for MU-Corn: ISU-Corn, MU-Rough: ISU-Byp., or MU-Rough: ISU-Corn across FE

classifications. Interestingly, slopes for LFE steers fed MU-Corn: ISU-Byp. were different from zero, estimating a decrease of 15 units of calpastatin activity ($P = 0.002$), and an increase in the extent of d 2 troponin-T degradation by 1.81 units ($P = 0.04$) for every one-tenth improvement in FP G:F. Slope estimates for d 14 troponin-T were not different from zero ($P \geq 0.31$) for MU-Corn:ISU-Byp, MU-Corn: ISU-Corn, or MU-Rough:ISU-Byp between FE classifications. However, the slope for d 14 troponin-T degradation of HFE steers receiving MU-Rough: ISU-Corn suggests the extent of troponin-T degradation decreases by 1.12 units ($P = 0.04$) as G:F increases by one-tenth in this treatment combination.

Discussion

Feed efficiency of livestock is influenced by many biological processes; of particular interest is the contribution of differences in protein turnover and tissue metabolism to the individual variation among animals. Steers in the present study showed extensive differences in RFI values, representing phenotypic extremes greater than 1.40 SD from the mean of the larger contemporary group from which they were selected. Calpastatin activity in the present study tended to be greater in steaks from HFE steers. In agreement with the current study, McDonagh et al. (2001) reported utilizing divergent selection for RFI for a single generation resulted in highly feed efficient steers having approximately 13% greater calpastatin activity compared to lowly feed efficient steers. However, no differences were noted in shear force of steaks from highly or lowly feed efficient steers. Cruzen et al. (2013b) reported that gilts selected for low RFI (greater efficiency) exhibit decreased calpain autolysis and increased calpastatin activity in muscle in comparison to high RFI (less efficient) gilts, suggesting protein degradation is decreased in highly efficient pigs. In contrast, Gomes et al. (2012), utilizing Nellore steers

classified as phenotypic extremes for RFI, reported no effect of RFI classification on WBSF, calpain 1, or calpastatin activity. The lack of differences reported by Gomes et al. (2012) in comparison to steers used in the present study is likely a response of breed type. It has been well documented that beef from *Bos Indicus* influenced cattle is less tender than beef from *Bos Taurus* cattle, due to increased levels of calpastatin activity decreasing protein degradation (Whipple et al., 1990, Shackelford et al., 1991b, Duarte et al., 2013).

Increased calpastatin activity combined with the numerical trends for decreased calpain autolysis and troponin-T degradation observed in HFE steers used in the present study suggests the extent of protein degradation is lessened, potentially resulting in increased muscle protein accretion. This may provide a potential explanation as to why some animals are more efficient. Herd and Arthur (2009) estimate that approximately 37% of the variation in RFI is explained by protein turnover, tissue metabolism, and stress. It has been hypothesized that some cattle are more feed efficient in part due to increased net protein accretion and less extensive protein degradation, partially due to increased calpastatin activity. Goll et al. (1998) reported that high calpastatin activity decreases the rate of muscle protein turnover and therefore is associated with increased rates of skeletal muscle growth. Although increased calpastatin activity has implications for improved efficiency of protein accretion in the animal, effects of increased calpastatin activity on postmortem conversion of muscle to meat may not be favorable. High calpastatin activity decreases calpain activity in postmortem muscle negatively affecting meat tenderness by decreasing protein degradation (Shackelford et al., 1991a; Morgan et al., 1993; Goll et al., 1998). The current study noted numerical trends for decreased amounts of completely autolyzed calpain 1, and greater calpastatin activity within HFE steers; however, with adequate aging, the differences in tenderness were not detected, as indicated by similar d 14 WBSF values

across FE phenotypes. This suggests that steaks from HFE steers were of similar tenderness to those from LFE steers and a consumer would be unlikely to detect differences.

The troponin-T structural protein is an example of a protein that undergoes postmortem degradation, and is commonly measured as an indicator of myofibrillar protein degradation. Measurements conducted on samples aged for 2 d indicated no differences due to diet type or FE classification. However, an observed effect in d 14 troponin-T degradation due to ISU diet suggests the extent of protein degradation was affected by finishing phase diet with greater amounts of degradation in steaks from steers finished on ISU-Corn diet compared to steers receiving the ISU-Byp based diet, although there were no observed differences in WBSF values due to ISU-diet. Differences in fiber content of the FP diets were discernable; however, dietary levels of sulfur were not measured and typically diets with greater inclusions of distillers' grains potentially have greater sulfur levels. It was hypothesized that inclusions of distillers' grains in the ISU-Byp. may have contributed greater concentrations of sulfur altering the extent of troponin- T degradation. The work of Pogge et al. (2014b) suggests that as dietary sulfur levels are increased troponin-T degradation was decreased. Interestingly, steers grown on MU-Rough (4.01 kg) had greater WBSF values than those grown on MU-Corn (3.43 kg), with an additional 0.58 kg of force required to shear steaks from steers grown on a roughage based diet. The WBSF of beef steaks from cattle receiving the MU-Rough diet is near the threshold at which consumers determine a steak to be tough, at a value of 4.1 kg according to Huffman et al. (1996). It is unlikely that the differences noted due to GP diet were a result of ADG during that period because the ADG across FE classification for the growing period was 1.94 kg/d for both MU-Corn and MU-Rough. Duarte et al. (2013), in a 2 × 2 factorial utilized Angus and Nellore cattle fed diets with a roughage to concentrate ratio of 100:0 or 70:30, concluded that feeding regime

had no effect on WBSF or myofibril fragmentation index suggesting diet type does not affect postmortem proteolytic activity. Limited work has evaluated diet regimen (concentrate vs. roughage) despite the fact that the majority of cattle in the US feeding system are grown on roughage based diets and finished on grain based diets. Therefore, a refinement of our knowledge of factors influencing ultimate tenderness of beef is needed.

During the finishing phase cattle are transitioned to high concentrate diets. This shift may alter FE leading to alterations in meat tenderness. However, it has been well documented that steers classified as either highly or lowly feed efficient based off growing phase RFI will maintain the same FE classification when transitioned to finishing phase diets (Kelley et al., 2010, Durunna et al., 2011, Russell et al., 2016a). To date, no work has evaluated the effect of finishing phase G:F on meat tenderness. In the present study statistical power (n = 6) may be limiting and these data are preliminary. The slope estimations in the present study suggest that for LFE steers receiving diet 1 (MU-Corn: ISU-Byp) d 2 calpastatin was decreased by approximately 15 units for each one-tenth (i.e. 0.10 to 0.20) improvement in G:F; however, because a one-tenth improvement in FE is very unlikely late in the feeding period a more practical interpretation would be a decrease of 1.5 calpastatin units for every one-hundredth of an improvement in G:F (i.e. 0.15 to 0.16). The decrease in calpastatin activity supports the noted increase in d 2 troponin-T of 1.81 units for each one-tenth improvement in FE, because as calpastatin activity decreases there is less inhibition of calpain, resulting in greater degradation of protein. McDonagh et al. (2001) noted that highly feed efficient cattle have increased calpastatin activity; however, estimated slopes for highly FE cattle were not affected in the present study. Although, interesting to note, slope estimates of lowly FE steers receiving MU-Corn: ISU-Byp indicate that as FE improves calpastatin activity decreases and protein degradation increases as

indicated by d 2 troponin-T. This suggests dietary management within FE classification has potential to alter tenderness attributes. Interestingly, slope analysis of d 14 troponin-T for HFE steers receiving diet 4 (MU-Rough: ISU-Corn) suggests a decrease of 1.12 units as G: F improves. Although finishing phase FE effects on meat tenderness have not been studied directly, evaluations of altered growth rates on calpain system activity have been evaluated. Sazili et al. (2003) reported that steers with a faster growth rate had greater amounts of calpastatin and decreased amounts of calpain 1. Conversely, Thomson et al. (1997) reported no difference in the activity of calpastatin, calpain 1, calpain 2 in lambs with altered growth rates. The effects of altered growth rate and FE late in the feeding period are not well understood, thus more extensive work with a larger population is required to better understand the impact of finishing phase FE on meat tenderness.

Carcass characteristics were varied across the FE phenotypically extreme steers. Steers classified as LFE had a greater DP than HFE steers; this is likely a reflection of the tendency for increased REA noted for steers classified as LFE. This contradicts the results of a multi-year feed efficiency study conducted by Russell et al. (2016a) with the same dietary treatments as those used in this study, which noted that steers classified as HFE tend to have a larger REA than their LFE counterparts. As the steers in the present study represent only a subset of the steers utilized in the analysis of Russell et al. (2016a) it is likely that more highly efficient steers will trend towards having a larger REA than those classified as lowly efficient. In agreement with the results of Russell et al. (2016a), steers finished on ISU-Byp had larger REA than steers fed the ISU-Corn diet. In the present study steers grown and finished on a corn based diet have greater amounts of BF; however, contrary to our results the large study by Russell et al. (2016a) suggests that cattle grown on roughage and finished on a by-product based diet had greater amounts of

BF. Interestingly, marbling scores were greater in the LFE steers finished on ISU-Byp diet. Klopfenstein et al. (2008) reported that the energetic value for distiller's grains is greater than that of corn. The greater inclusion of distillers' grains in the ISU-Byp diet combined with the greater intakes in the LFE steers suggests there was more energy available to these steers, and as a result excess energy was retained and deposited as fat. Russell et al. (2016a) reported no interaction between ISU diet and FE classification, but reported that marbling scores decreased as feed efficiency increased in steers. Covington et al. (1970) found that marbling effects on tenderness are minimal, as differences in shear force between steaks with moderate or small degrees of marbling only differed by 0.37 kg, a value below the 0.5 kg threshold for detection by consumers.

Under the conditions of our study, minimal differences in meat tenderness were observed among steers identified as phenotypically extremes for high or low feed efficiency. This suggests that cattle producers can continue to select for improved feed efficiency with minimal concerns for ultimate meat tenderness implications for the consumer. However, our fundamental understanding of how feed efficiency and diet type affects components of the calpain system and underlying effects on meat quality is limited. Results of the present study suggest further research is needed with a larger population to understand the effect feeding regimen has on meat tenderness. Steers grown on more fibrous diets had greater variation in the extent of protein degradation producing steaks with slightly greater WBSF values, suggesting dietary fiber content may impact meat tenderness. Evaluation of management decisions to improve production efficiency in the beef industry, and the underlying effects on beef tenderness perception by consumers will help to support the long term sustainability of the industry.

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TABLES AND FIGURES

Table 1. Ingredient and nutrient composition of growing period diets fed to steers (76 d).

Ingredient, % DM	MU-Rough	MU-Corn
Whole shell corn	-	64.26
Soybean hull pellets	36.84	-
Sudan baleage	36.25	-
DDGS[1]	22.70	26.07
Soyplus[2]	1.75	4.96
Porcine blood meal	1.65	2.52
Limestone	0.35	1.09
Urea	-	0.19
Salt	0.18	0.22
Vitamin premix[3]	0.18	0.23
Trace mineral premix[4]	0.07	-
MFP[5]	0.03	
Pellet binder	-	0.19
Rumensin 90[6]	0.01	0.01
Nutritional analysis[7]		
DM. % as-fed basis	66.8	85.1
NDF, % DM	57.5	26.4
ADF, % DM	31.5	6.5
CP, % DM	20.8	20.5

[1] DDGS: Dried distillers grains plus solubles

[2] Soyplus (West Central Cooperative, Ralston, IA)

[3] Vitamin premix contains 2,200 IU vitamin A, 275 IU vitamin D, 100 IU vitamin E per kg of diet

[4] Trace mineral premix offered 10 mg Cu, 50 mg Fe, 20 mg Mn, 30 mg Zn, 0.1 mg Co, 0.1 mg Se, 0.5 mg I per kg diet

[5] DL-methionine hydroxyanalogue calcium (84% methionine, Novus International, Saint Charles, MO)

[6] Provided Monensin at 150 mg· steer^{-1} · d^{-1}, Elanco Animal Health, Indianapolis, IN

[7] Determined from analysis of total mixed rations

Table 2. Ingredient and nutrient composition of diets fed to steers during the finishing period (87 d).

Ingredient, % DM	ISU-Corn[1]	ISU-Byp[1]
Cracked corn	75	30
DDGS[2]	14.99	39.99
Soybean hull pellets	-	20
Bromegrass hay	8	8
Limestone	1.54	1.54
Salt	0.31	0.31
Vitamin A premix[3]	0.11	0.11
Trace mineral premix[4]	0.035	0.035
Rumensin 90[5]	0.013	0.013
Nutritional analysis[6]		
DM, % as-fed basis	84.5	84.1
NDF, % DM	24.4	42.7
ADF, % DM	8.0	18.7
CP, % DM	11.2	18.4

[1] Finishing phase diets: ISU Corn = cracked corn-based; ISUByp = dried distillers grains and soybean hull-based.

[2] DDGS: Dried distillers grains plus solubles
Vitamin A premix contained 4,400,000 IU/kg.

[3] Vitamin A premix contained 4,400,000 IU/kg.

[4] Provided per kilogram of diet (from inorganic sources): 30 mg Zn, 20 mg Mn, 0.5 mg I, 0.1 mg Se, 10 mg Cu, 0.1 mg Co.

[5] Provided Monensin at 200 mg·steer^{-1}·d^{-1}, Elanco Animal Health, Indianapolis, IN.

[6] Determined from analysis of total mixed rations.

Table 3. Summary statistics of the greatest and least feed efficient steers during the growing period[1]

	Growing phase diets[2]			
	MU- Corn		MU-Rough	
	Growing phase feed efficiency classifications[3]			
Item	LFE	HFE	LFE	HFE
Steers, *n*	12	12	12	12
Average RFI	2.98	-3.04	2.83	-3.62
SD from mean[4]	1.61	-1.64	1.42	-1.82
Average G:F	0.215	0.291	0.190	0.267
SD[5]	0.026	0.035	0.022	0.013
Average ADG, kg/d	2.00	1.87	1.94	1.95
SD[5], kg/d	0.256	0.258	0.387	0.317
Average DMI, kg/d	9.34	6.46	10.34	7.31
SD[5], kg/d	0.816	0.753	1.174	1.184

[1] Steers identified as greatest and least efficient from total growing period groups: MU-Corn = 90 steers; MU-Rough = 91

[2] Growing phase diets: MU-Corn = whole shell-corn based; MU-Rough = sudan baleage and soybean hull-based

[3] Growing period feed efficiency classifications: LFE = least feed efficient; HFE = most feed efficient

[4] Average SD from the RFI mean of the total groups fed each growing phase diet

[5] Standard deviation for feed efficiency classification within growing phase diet

Table 4. Evaluation of growing and finishing phase diet type and feed efficiency classification on the carcass characteristics of beef steers.

	MU Diet[1]							
	Corn		Rough					
	ISU Diet[2]					*P* values		
Item[2]	Corn	Byp.	Corn	Byp.	SEM	MU Diet	ISU Diet	MU Diet × ISU Diet
HCW, kg	385	382	365	389	8.30	0.41	0.20	0.11
BF, cm	1.43[a]	1.19[b]	0.88[c]	1.22[b]	0.075	0.0014	0.53	0.0003
KPH, %	2.46	2.38	2.29	2.42	0.074	0.40	0.78	0.17
Dressing[3], %	60.8[c]	63.2[a]	61.6[bc]	62.0[b]	0.395	0.65	0.0014	0.014
Yield grade[4]	2.67[a]	2.0[b]	1.92[b]	2.08[b]	0.121	0.009	0.046	0.001

[abc] Least squares means in a row without common superscripts differ ($P \leq 0.05$)
[1] MU Diet: growing phase diet either MU-Corn or. MU-Rough
[2] ISU Diet: finishing phase diet either ISU-Byp. or. ISU-Corn
[2] MU Diet × ISU Diet × feed efficiency classification (FE), ($P \geq 0.41$)
[3] Main effect of FE, ($P = 0.012$), lowly FE: 62.4, highly FE: 61.4, SEM = 0.280
[4] ISU Diet × feed efficiency ($P = 0.045$), ISU-Corn –LFE: 2.1, ISU-Corn-HFE: 2.5, ISU-Byp- LFE:

Table 5. Evaluation of growing and finishing phase diet type and feed efficiency classification on Minolta objective color scores, percent cook loss, proximate analysis, protein expression and WBSF of steaks.

	MU diet[1]		ISU diet[2]		FE[3]			*P* values[1]		
Item[4]	Corn	Rough	Corn	Byp	High	Low	SEM	MU Diet	ISU Diet	FE
Calpain 1[5]	77.2	75.2	77.1	75.3	74.13	78.22	2.00	0.48	0.53	0.15
D 2 troponin-T[6]	39.9	32.5	39.7	32.7	29.98	42.44	6.79	0.44	0.47	0.20
D 14 troponin-T[6]	73.5	84.9	89.9	68.4	73.58	84.73	7.26	0.12	0.005	0.13
WBSF, kg	3.43	4.01	3.55	3.89	3.67	3.77	0.202	0.05	0.24	0.74
Minolta L*[7]	34.9	35.1	34.6	35.4	34.6	35.4	0.47	0.86	0.21	0.25
Minolta a*[8]	19.2	19.1	19.2	19.1	19.2	19.0	0.19	0.63	0.73	0.44
Minolta b*[9]	4.9	4.8	4.8	4.9	4.7	4.9	0.12	0.76	0.60	0.23
Cook loss,%	13.9	13.7	13.8	13.7	13.6	13.9	0.27	0.62	0.89	0.41
Moisture, %	70.1	70.2	70.1	70.2	70.6	69.7	0.37	0.78	0.86	0.16
Protein, %	23.1	23.0	23.0	23.1	23.1	23.1	0.16	0.76	0.89	0.95

[1] MU Diet: MU-Corn vs. MU-Rough;
[2] ISU Diet: ISU-Byp. vs. ISU-Corn
[3] FE Classification: Highly efficient vs Lowly efficient
[4] No three-way interaction $P \geq 0.12$; two-way interaction $P \geq 0.19$
[5] Calpain 76: reported as the percentage of completely autolyzed calpain 1 (76 kDa) measured on samples aged 2 d
[6] Troponin-T: reported as percentage of troponin-T degradation product relative to a pooled sample from the same day
[7] Measure of lightness: 0: black and 100: white
[8] Measure of redness, + values: red, - values: green
[9] Measure of yellowness, + values: yellow, - values: blue

Table 6. Summary statistics of the greatest and least feed efficient steers during the finishing period within diet combination.[1]

	Growing phase diets[2]							
	MU- Corn				MU-Rough			
	HFE[3]		LFE[3]		HFE[3]		LFE[3]	
	Finishing phase diets[4]							
Item	Corn	Byp.	Corn	Byp.	Corn	Byp.	Corn	Byp.
Steers, *n*	6	6	6	6	6	6	6	6
Average G:F	0.172	0.194	0.161	0.176	0.175	0.189	0.182	0.170
SD[5]	0.022	0.025	0.021	0.020	0.022	0.013	0.024	0.037
Average ADG, kg/d	1.61	1.86	1.80	1.86	1.71	1.90	1.71	1.90
SD[5], kg/d	0.35	0.44	0.24	0.13	0.31	0.16	0.23	0.49
Average DMI, kg/d	9.32	9.52	11.32	10.68	9.71	10.16	9.39	11.06
SD[5], kg/d	1.46	1.20	1.50	1.00	1.09	1.40	0.67	1.21

[1] Steers identified as greatest and least efficient from total growing period groups: MU-Corn = 90 steers; MU-Rough = 91
[2] Growing phase diets: MU-Corn: whole shell-corn based; MU-Rough: sudan baleage and soybean hull-based
[3] Growing period feed efficiency classifications: LFE: least feed efficient; HFE: most feed efficient
[4] Finishing phase diets: IUS-Corn: cracked corn based; ISU-Byp: soyhull based diet
[5] Standard deviation for feed efficiency classification within diet combination

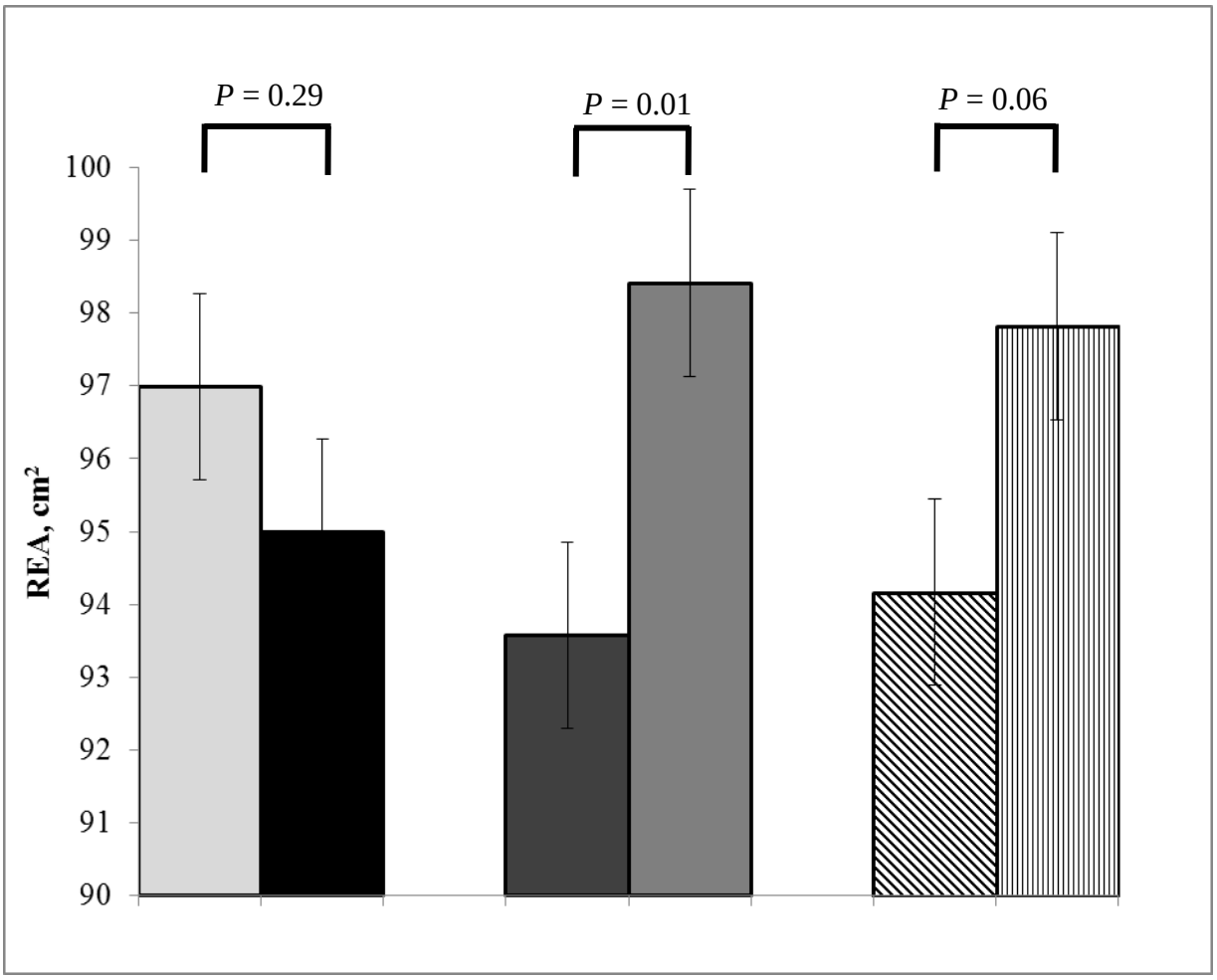
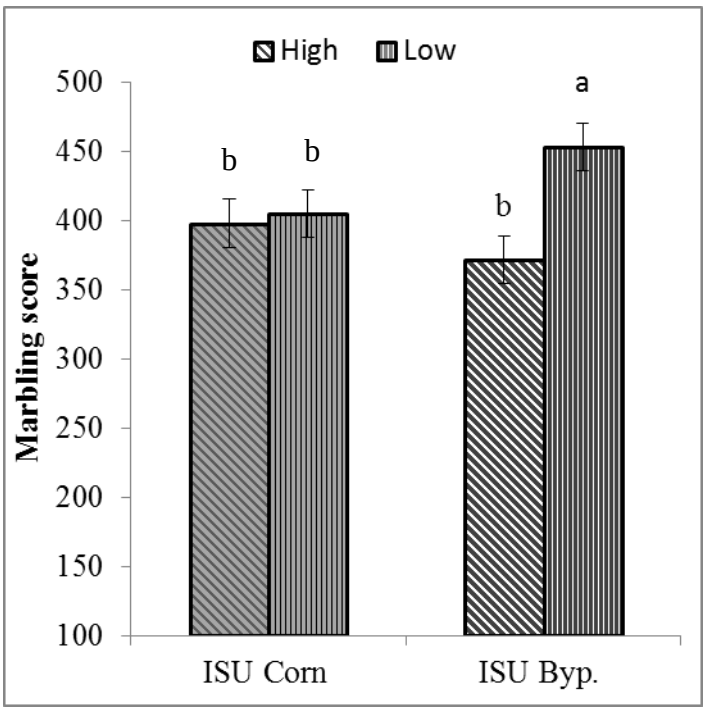


Figure 1. The effect of growing (MU- Corn; MU- Rough) and finishing phase (ISU- Corn, ISU-Byp) diet type and growing phase feed efficiency (HFE; LFE) classification on REA of beef steers.

A.



B.

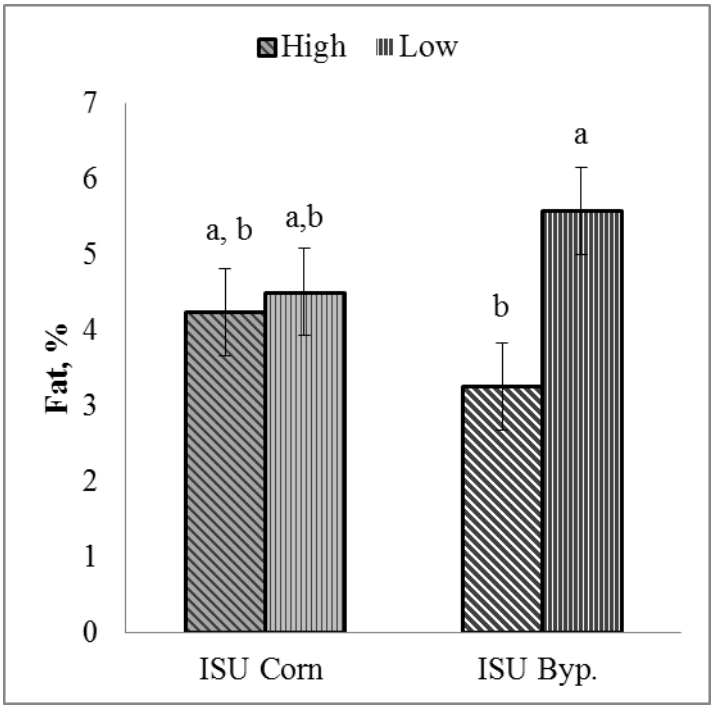


Figure 2. The effect of growing phase feed efficiency on marbling score (A) and fat content (B) of steaks from steers finished on corn (ISU-Corn) or byproduct (ISU-Byp) based diets. ISU diet × feed efficiency; $P = 0.04$ for marbling score and $P = 0.08$ for fat content. Within a panel columns with differing superscripts are different ($P \leq 0.05$).(A): Marbling score equivalents: 300 = Slight, 400 = Small, 500 = Modest, 600 = Moderate. (B): Percentage of fat present in a sub sample of longissimus dorsi muscle.

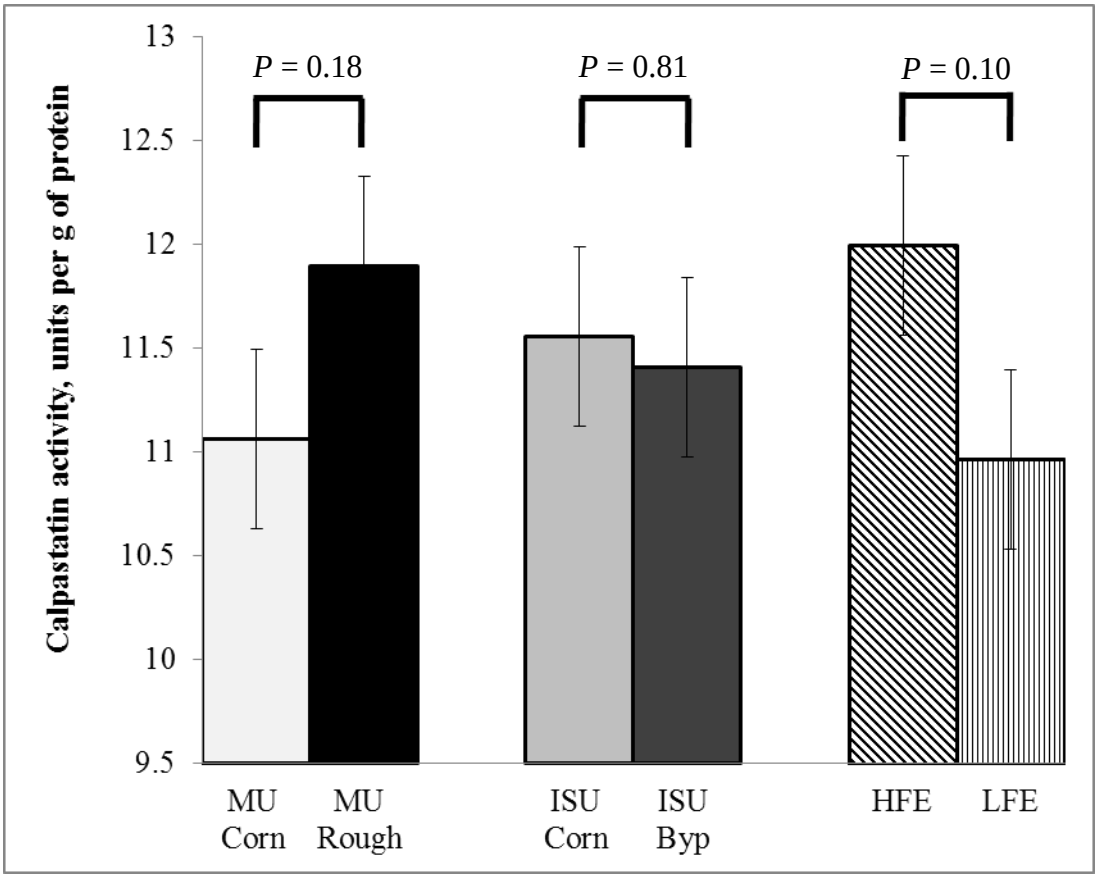
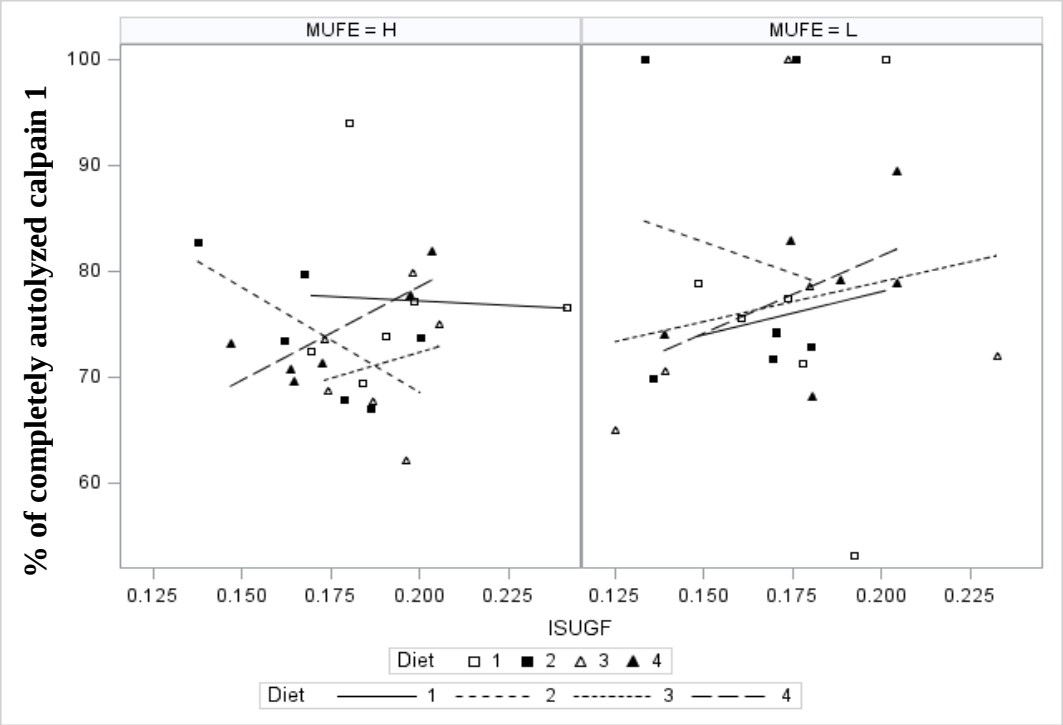


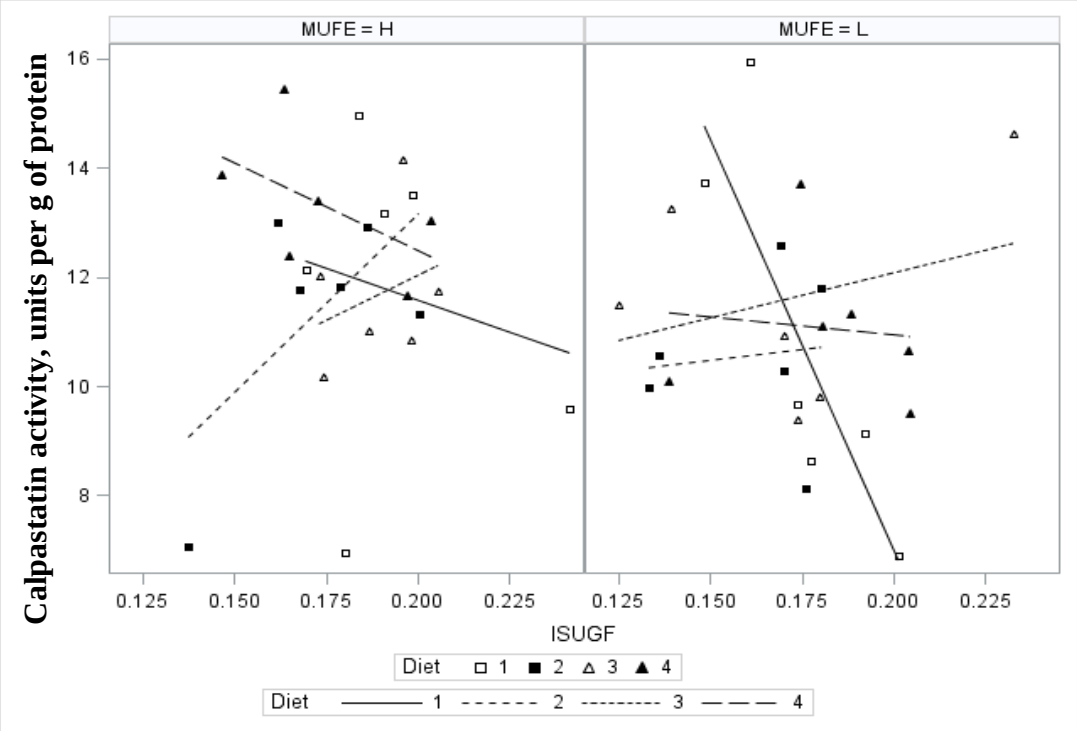
Figure 3. The effect of growing (MU- Corn; MU- Rough) and finishing phase (ISU- Corn, ISU-Byp) diet type and growing phase feed efficiency (HFE; LFE) classification on d 2 calpastatin activity of steaks.



FE[1]	Diet	MU Diet[2]	ISU Diet[3]	Slope Estimate	SEM	*P*-value
H	1	Corn	Byp	-1.66	19.21	0.93
H	2	Corn	Corn	-19.81	22.34	0.38
H	3	Rough	Byp	9.85	36.52	0.79
H	4	Rough	Corn	17.74	22.34	0.43
L	1	Corn	Byp	8.14	24.84	0.75
L	2	Corn	Corn	-11.85	23.36	0.62
L	3	Rough	Byp	7.56	12.95	0.56
L	4	Rough	Corn	14.66	19.90	0.47

[1] Growing phase feed efficiency: H: highly efficient; L: lowly efficient
[2] MU-diet: growing phase diet either MU-Corn or MU- Rough
[3] ISU diet: finishing phase diet either ISU- Corn or ISU-Byp.

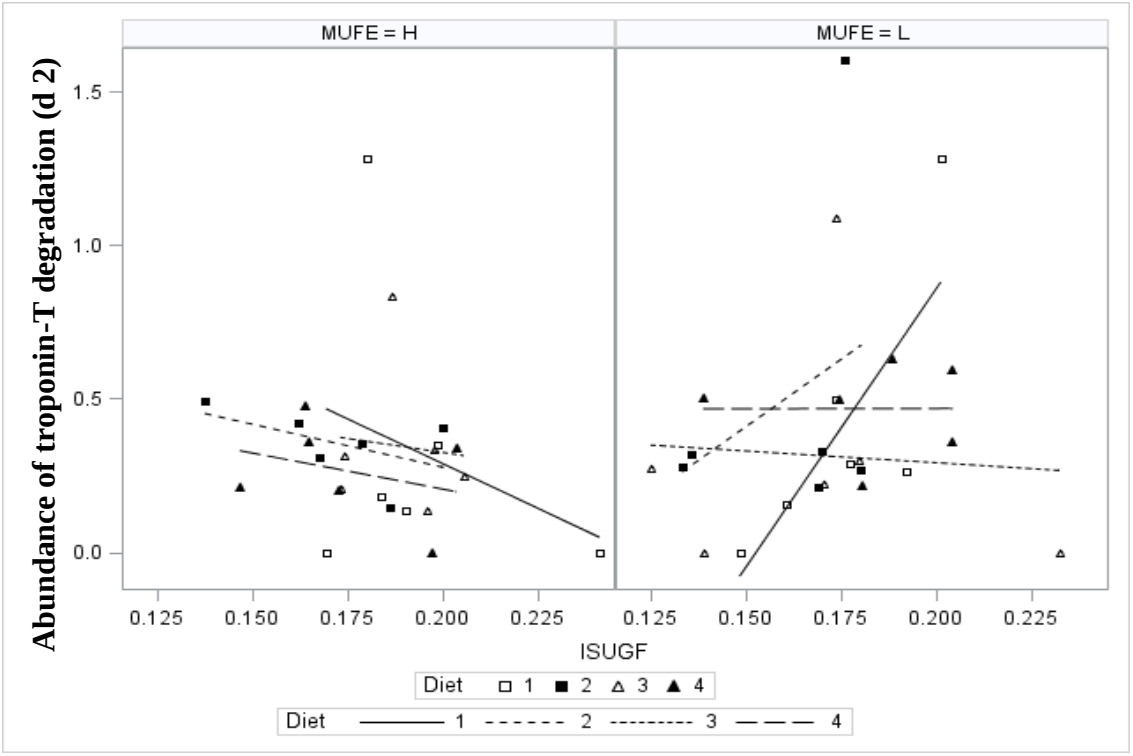
Figure 4. Effect of finishing phase feed efficiency (G:F) on completely autolyzed calpain 1 (76 kDa) measured on d 2 in Exp. 2. Diet combinations are the following: Diet 1: MU-Corn: ISU-Byp; Diet 2: MU-Corn: ISU- Corn; Diet 3: MU-Rough: ISU-Byp; MU-Rough: ISU-Corn.



FE[1]	Diet	MU Diet[2]	ISU Diet[3]	Slope Estimate	SEM	*P*-value
H	1	Corn	Byp	-2.32	3.53	0.51
H	2	Corn	Corn	6.53	4.11	0.12
H	3	Rough	Byp	3.30	6.72	0.63
H	4	Rough	Corn	-3.21	4.11	0.44
L	1	Corn	Byp	-15.04	4.57	0.002
L	2	Corn	Corn	0.79	4.30	0.85
L	3	Rough	Byp	1.65	2.38	0.49
L	4	Rough	Corn	-0.66	3.66	0.86

[1] Growing phase feed efficiency: H: highly efficient; L: lowly efficient
[2] MU-diet: growing phase diet either MU-Corn or MU- Rough
[3] ISU diet: finishing phase diet either ISU- Corn or ISU-Byp.

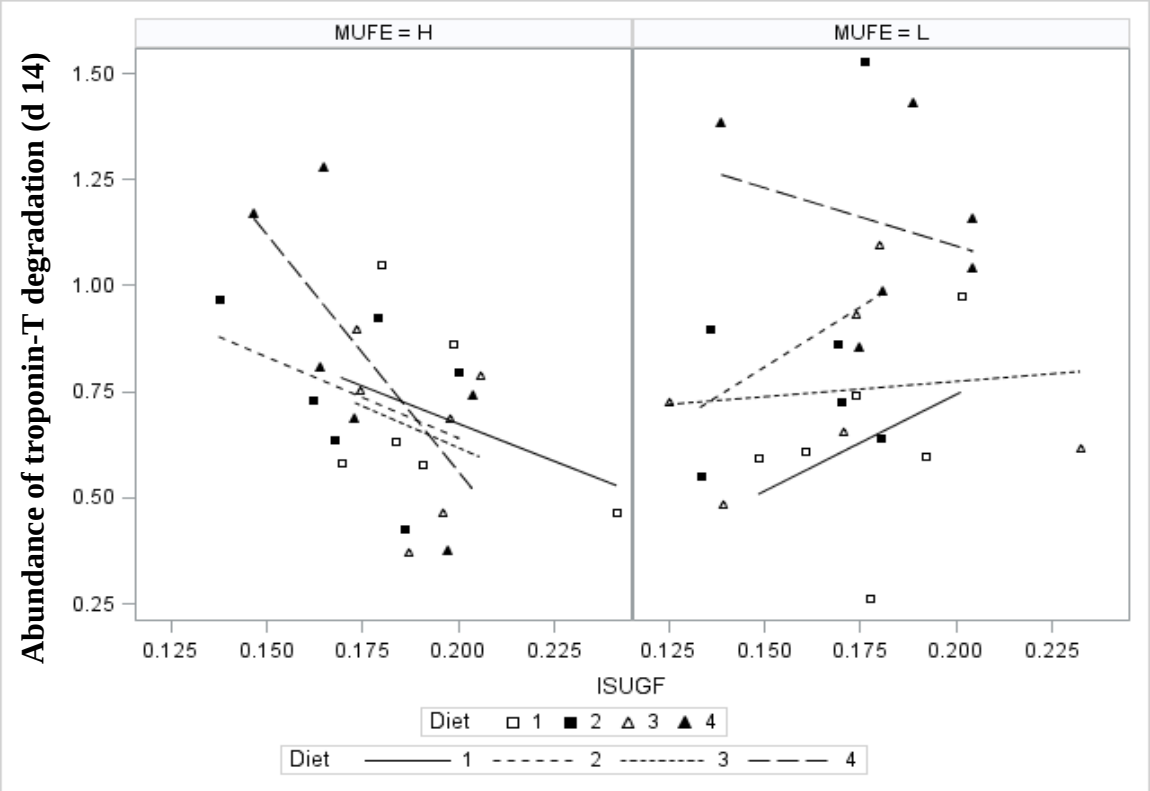
Figure 5. Effect of finishing phase feed efficiency (G:F) on d 2 calpastatin activity per gram of protein in Exp. 2. Diet combinations are the following: Diet 1: MU-Corn: ISU-Byp; Diet 2: MU-Corn: ISU- Corn; Diet 3: MU-Rough: ISU-Byp; MU-Rough: ISU-Corn.



FE[1]	Diet	MU Diet[2]	ISU Diet[3]	Slope Estimate	SEM	*P*-value
H	1	Corn	Byp	-0.58	0.64	0.37
H	2	Corn	Corn	-0.28	0.75	0.71
H	3	Rough	Byp	-0.18	1.22	0.88
H	4	Rough	Corn	-0.23	0.75	0.76
L	1	Corn	Byp	1.81	0.83	0.04
L	2	Corn	Corn	0.88	0.78	0.27
L	3	Rough	Byp	0.076	0.43	0.86
L	4	Rough	Corn	0.0019	0.66	1.0

[1] Growing phase feed efficiency: H: highly efficient; L: lowly efficient
[2] MU-diet: growing phase diet either MU-Corn or MU- Rough
[3] ISU diet: finishing phase diet either ISU- Corn or ISU-Byp.

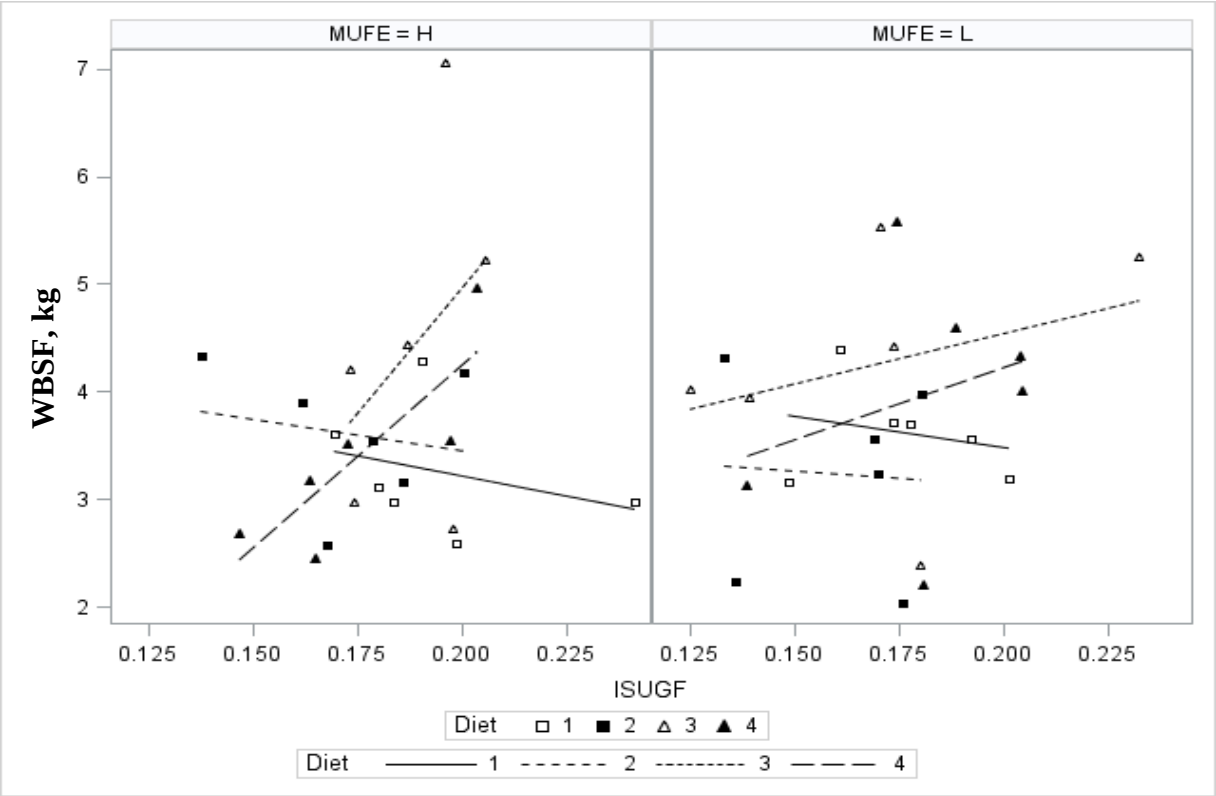
Figure 6. Effect of finishing phase feed efficiency (G:F) on abundance of d 2 troponin-T degradation product in Exp. 2. Diet combinations are the following: Diet 1: MU-Corn: ISU-Byp; Diet 2: MU-Corn: ISU- Corn; Diet 3: MU-Rough: ISU-Byp; MU-Rough: ISU-Corn.



FE[1]	Diet	MU Diet[2]	ISU Diet[3]	Slope Estimate	SEM	*P*-value
H	1	Corn	Byp	-0.35	0.45	0.44
H	2	Corn	Corn	-0.38	0.52	0.47
H	3	Rough	Byp	-0.39	0.86	0.65
H	4	Rough	Corn	-1.12	0.52	0.04
L	1	Corn	Byp	0.45	0.58	0.44
L	2	Corn	Corn	0.56	0.55	0.31
L	3	Rough	Byp	0.072	0.30	0.81
L	4	Rough	Corn	-0.27	0.47	0.56

[1] Growing phase feed efficiency: H: highly efficient; L: lowly efficient
[2] MU-diet: growing phase diet either MU-Corn or MU- Rough
[3] ISU diet: finishing phase diet either ISU- Corn or ISU-Byp.

Figure 7. Effect of finishing phase feed efficiency (G:F) on abundance of d 14 troponin-T degradation product in Exp. 2. Diet combinations are the following: Diet 1: MU-Corn: ISU-Byp; Diet 2: MU-Corn: ISU- Corn; Diet 3: MU-Rough: ISU-Byp; MU-Rough: ISU-Corn.



FE[1]	Diet	MU Diet[2]	ISU Diet[3]	Slope Estimate	SEM	*P*-value
H	1	Corn	Byp	-0.74	1.80	0.68
H	2	Corn	Corn	-0.58	2.09	0.78
H	3	Rough	Byp	4.65	3.42	0.18
H	4	Rough	Corn	3.40	2.09	0.11
L	1	Corn	Byp	-0.58	2.33	0.80
L	2	Corn	Corn	-0.27	2.19	0.90
L	3	Rough	Byp	0.94	1.21	0.44
L	4	Rough	Corn	1.34	1.86	0.48

[1] Growing phase feed efficiency: H: highly efficient; L: lowly efficient
[2] MU-diet: growing phase diet either MU-Corn or MU- Rough
[3] ISU diet: finishing phase diet either ISU- Corn or ISU-Byp.

Figure 8. Effect of finishing phase feed efficiency (G:F) on WBSF of steaks aged 14 d in Exp.2. Diet combinations are the following: Diet 1: MU-Corn: ISU-Byp; Diet 2: MU-Corn: ISU- Corn; Diet 3: MU-Rough: ISU-Byp; MU-Rough: ISU-Corn

CHAPTER 4.

EFFECT OF POST-ETHANOL EXTRACTION SORGHUM SILAGE AS A FORAGE SOURCE IN GROWING AND FINISHING DIETS ON STEER PERFORMANCE, CARCASS CHARACTERISTICS AND NUTRIENT DIGESTIBILITY

A paper to be submitted to the *Journal of Animal Science*

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Abstract

Two experiments evaluated the use of post-ethanol extraction sorghum silage as an alternative forage source in feedlot diets. Seventy-two crossbred steers (397 kg ± 23, SD) were used to evaluate growth and carcass characteristics. Steers were blocked by BW into pens of 6 steers and randomly assigned to growing diets containing 40% (DM basis) of sorghum silage (**SS;** 57.6% NDF) or grass hay (**CON;** 63.3% NDF) for 56 d (*n* = 6 pens per treatment). Within each treatment steers transitioned to dry-rolled corn-based finishing diets (fed for 56 d) containing 6% effective NDF contributed by the forage source, resulting in forage inclusions of 16% for SS and 13.1% for CON, where forage replaced corn. A sub-sample of steers (*n* =12 per treatment) housed in pens equipped with GrowSafe bunks were used for determination of growing phase diet total tract digestibility. From d 28 to 42, steers received titanium dioxide at approximately 10 g^{-1}·$steer^{-1}$·day^{-1}, and fecal samples were collected on d 41 and 42. Fecal and total mixed ration samples were dried, and ground for analysis of DM, OM, NDF, ADF, CP,

ether extract (**EE**), and starch. Data were analyzed using PROC MIXED of SAS with the fixed effects of treatment and block for performance and carcass data or treatment for digestibility data; significance was determined at $P \leq 0.05$ and tendencies at $P \leq 0.10$. Growing phase DMI and ADG did not differ due to treatment ($P \geq 0.19$); however, SS-fed steers had improved G:F compared to CON ($P = 0.04$). Finishing period ADG and G:F did not differ ($P \geq 0.15$) between treatments, despite SS-fed steers having lesser ($P = 0.008$) DMI than CON-fed steers. No differences in DMI, ADG, or G:F over the whole trial were noted between treatments ($P \geq 0.12$), nor were any carcass traits affected ($P \geq 0.23$). Growing phase total tract apparent digestibility of DM and starch did not differ ($P \geq 0.19$) due to treatment; however, OM digestibility tended to be greater ($P = 0.09$) in SS-fed steers. During the digestibility assessment period DMI was lesser ($P = 0.003$) in SS-fed steers. Steers fed the SS diet had greater ($P \leq 0.03$) digestibility of EE, CP, NDF, and hemicellulose than CON-fed steers. However, CON-fed steers had greater ($P < 0.0001$) ADF digestibility than SS-fed steers. These data suggest that post-extraction sorghum silage can be effectively utilized in feedlot diets as alternative forage source.

Key words: cattle performance, digestibility sorghum silage

Introduction

Roughage is a critical component in ruminant diets, functioning to maintain proper health of the rumen (Allen, 1997). A high forage diet, typical of those fed during the growing phase in the U.S beef industry commonly consists of higher quality forages which provide a significant amount of nutrients for growing cattle. Use of sorghum silages in feedlot diets has been limited in previous years due to lower digestibility as a result of greater lignin content than traditional

forages making it less desirable as a roughage source in feedlot diets. However, advancements in sorghum genetics have introduced hybrid cultivars reported to contain lesser amounts of lignin and thus are more digestible than grain cultivars (Houx et al., 2013). Sweet sorghum cultivars, with increased sugar content, have been evaluated for use in bio-fuel production (Smith et. al., 1987; Putnam et al., 1991; Rooney et al., 2007). Post-processing residues may then hold value for use as ruminant feedstuffs. This post-extraction sorghum silage by-product has a nutrient profile with a high fiber content which makes it a viable replacement for forage sources such as hay or corn silage in ruminant diets. The objectives of this study were to evaluate post-extraction sorghum silage as an alternative to hay in feedlot diets on growing phase diet digestibility and growing and finishing phase growth and feed efficiency of beef steers.

Materials and methods

All procedures involving the use of animals were approved by the Institutional Animal Care and Use Committee of Iowa State University (#4-15-8008-B).

Animals and experimental design

Seventy-two Angus-cross steers were purchased from a single source and transported to the Iowa State University Beef Nutrition Farm (Ames, IA). Upon arrival all cattle were dewormed with Ivomec Eprinex Pour-On (Merial Animal Health, Duluth, GA), vaccinated with Bovi-shield GOLD 5 (Zoetis, New York, NY), and assigned individual visual and electronic identification tags. Steers were fed a receiving diet for 7 d. Prior to the trial forages were analyzed for nutrient composition at Dairyland Laboratories Inc. (Arcadia, WI). All post extraction sorghum silage was produced at a single time to minimize variation and was shipped

to Iowa State University in 1.2 × 0.91 m plastic, airtight shipping containers as needed during the trial. The nutrient profile of sorghum silage included: 8.7% CP, 57.6% NDF, 40.5% ADF, and 2.5% ether extract (**EE**). The hay used in this trial was predominately bromegrass with some red clover. The nutrient profile of the hay fed was: 13.3% CP, 63.3% NDF, 42.9% ADF, and 3.0% EE. At initiation of the trial, steers were implanted with Component TE-IS with Tylan [80 mg trenbolone acetate, 16 mg estradiol, and 29 mg tylosin tartrate; Elanco Animal Health, Greenfield, IN]; individual weights were taken on two consecutive days and steers were blocked by initial BW (396 ± 23.7 kg) into pens (6 steers/pen and 6 pens/treatment). Pens within block were randomly assigned to growing phase diets (**Table 1**) including 40% hay (DM basis) control diet (**CON**) or a 40% sorghum-silage (DM basis) diet (**SS**); fed for 56 d. Of the 6 pens per treatment 4 had traditional fence line cement bunks and 2 had GrowSafe equipped-bunks (GrowSafe Systems Ltd., Airdrie, AB Canada) to allow for the measurement of individual feed intake. For the duration of the trial the two middle weight blocks of steers were selected to be housed in GrowSafe-equipped pens. These steers were previously allowed to acclimate to the GrowSafe-equipped pens for 7 d before the start of the trial. All steers were transitioned to finishing diets using three step-up diets from d 56 through d 76. Forage content was 30, 20, and 13.1% of diet DM in step-up diet 1, 2, and 3, respectively, for hay and 30, 20, and 16% for sorghum silage step-up diets 1, 2, and 3. Steers received a Component TE-S implant with Tylan [120 mg trenbolone acetate, 24 mg estradiol, and 29 mg tylosin tartrate; Elanco Animal Health] at the beginning of the finishing phase, and were fed corn-based finishing diets for 56 days (**Table 1**). Effective neutral detergent fiber (eNDF), defined as the amount of NDF equal to or greater than 7.87 mm, was calculated for each forage source using a Penn State particle separator. Concentrations of eNDF were 45.8% and 37.5% for hay and sorghum silage,

respectively. During the finishing phase steers continued to receive the same forage as during the growing phase, but at a decreased inclusion, balanced to offer 6% eNDF to the diet from each forage source, with forage displacing corn in the diets. Additional BW were collected on d 28, 55, 56, 76, 77, and 105. Final body weights were collected on 2 consecutive days prior to harvest (d 132 and 133).

Sample collection and analytical procedures

Feed was delivered to steers once daily at approximately 0700 h, and cattle were allowed ad libitum access to water. Total feed delivered and bunk scores were recorded daily according to the slick bunk management protocol as described by Drewnoski et al. (2014). Diet components and total mixed rations were sampled weekly to determine DM content. Orts (feed refusals) were weighed and sampled on cattle weigh dates. Feed and ort samples were dried in a forced air oven at 70°C for 48 h and used to calculate feed efficiency (G:F) for each weigh period from pen DMI and average weight gain per pen. Steers housed in GrowSafe equipped pens (*n* = 12 steers per treatment, 2 pens per treatment) were utilized to determine diet total tract digestibility during the growing phase. Titanium dioxide, an indigestible marker, was offered at approximately 10 g^{-1}•$steer^{-1}$• day^{-1} for 14 d and fecal samples were collected on day 42 and 43. Total mixed ration samples were collected on d 0, 7, and 14 of the titanium dioxide feeding period and diet nutrient composition during this period is shown in **Table 2**. Diet and fecal samples were analyzed for DM, OM, NDF, ADF, CP, and EE as previously described by Lundy et al. (2015). Starch sample preparation was performed using the methods of Russell et al. (2016) and analyzed according to the procedures of the Megazyme D-glucose assay (Megazyme International Ireland, Co. Wicklow, Ireland). Samples for the titanium dioxide analysis were

prepared using the method of Myers et al. (2004) and were analyzed for titanium dioxide concentrations calorimetrically (Eon Microplate Spectrophotometer, BioTek, Winooski, VT). Fecal output was then estimated based on titanium dioxide intake divided by fecal titanium concentration. Fecal output and fecal nutrient concentrations were multiplied to calculate individual steer nutrient output. Likewise, diet nutrient composition was multiplied by individual steer intake to determine nutrient intake. These data were used to calculate percent nutrient digestibility, using the equation:

$$[(\text{diet nutrient intake} - \text{fecal nutrient output}) \div (\text{diet nutrient intake})] \times 100.$$

Kilograms of each nutrient digested were calculated as diet nutrient intake – fecal nutrient output. Steers were shipped to a commercial abattoir (Iowa Premium Beef, Tama, IA) on d 133, and individual animal identification remained with each carcass post-harvest. Carcasses were chilled for 48 h before being ribbed between the 12th and 13th ribs and graded by a USDA grader. Carcass data collected from all steers included: HCW, back fat (BF), ribeye area (REA), KPH, marbling score, yield grade (YG) and quality grade (QG). A 4% pencil shrink was applied to all live BW measurements before calculation of performance data.

Statistical analysis

Live animal performance and carcass data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) as a randomized complete block design with the fixed effects of treatment and block. Pen served as the experimental unit for BW, DMI, ADG, G:F, and carcass data analysis (n = 6 per treatment). For nutrient digestibility data determined using steers fed in GrowSafe equipped-bunks steer was the experimental unit (n = 12 per treatment). Data reported

are LSMeans and SEM. Significance was declared at $P \leq 0.05$ and tendencies from $0.06 < P \leq 0.10$.

Results

Live steer performance and carcass characteristics

Steer performance results are presented in **Table 3**. During the growing period (d 0 to 56) there were no differences noted in DMI or ADG ($P \geq 0.19$); however, there was a difference in G:F ($P = 0.04$), with SS-fed steers having better feed efficiency than CON-fed steers. During the finishing phase (d 77 to 133) SS-fed steers consumed less DM ($P = 0.008$) than controls, but there were no differences observed in ADG, G:F, or final BW ($P \geq 0.15$). Overall performance for the duration of the trial (d 0 to 133), showed no differences for DMI, ADG, or G:F between diets ($P \geq 0.12$). Similarly, carcass characteristic data (**Table 4**) were not affected by treatment as no differences were noted in HCW, DP, BF, KPH, REA, YG, marbling score, and QG.

Growing period nutrient digestibility

Diet nutrient digestibility data are presented in **Table 5** and amounts of digested nutrients are presented in **Table 6**. Digestibility of DM was similar between diets ($P = 0.19$); however, steers fed the SS diet digested less total DM ($P = 0.04$) because they had lesser DMI ($P = 0.003$) during the titanium feeding period. Sorghum silage-fed steers tended ($P = 0.09$) to have improved OM digestibility, though total OM digested did not differ due to treatment ($P = 0.15$). Digestibility of NDF was increased in steers fed SS ($P = 0.02$) compared with CON-fed steers; however, ADF digestibility was decreased by approximately 14% in steers fed the SS diet ($P < 0.0001$) compared with controls. These data indicate a difference in hemicellulose digestibility,

calculated by the difference in NDF and ADF fractions of the diet, with an approximately 18% greater digestibility of the hemicellulose fraction of fiber in cattle fed SS ($P < 0.0001$). Diet nutrient disappearance shows a similar trend, NDF disappearance was greater and ADF disappearance was lesser in SS-fed steers ($P < 0.0001$), and calculated hemicellulose disappearance was greater in the SS-fed steers ($P < 0.0001$). Cellulose digestibility was greater in SS-fed steers with approximately 7% ($P = 0.03$) advantage over CON-fed steers, and steers fed the SS diet also had greater total cellulose disappearance ($P < 0.0001$). Digestibility of CP was 3% greater for steers fed SS in comparison to CON-fed steers ($P = 0.001$), but interestingly there is greater disappearance of CP in CON-fed steers ($P < 0.0001$), because samples collected during the digestibility portion of the trial analyzed to contain more CP in CON samples (15.6%) vs. SS samples (13.7%) despite diets being formulated to contain equal amounts of CP. Ether extract digestibility follows a similar trend with SS-fed cattle having approximately 7% greater digestibility than CON-fed cattle ($P < 0.0001$) but EE disappearance was similar between treatments ($P = 0.38$). Digestibility of starch did not differ due to treatment ($P = 0.26$); however, because of the greater analyzed starch content of the SS diet there is increased disappearance of starch in steers fed the SS diet relative to steers fed CON ($P < 0.0001$)

Discussion

The use of agricultural commodities for the production of alternative energy fuels, like ethanol, has greatly expanded in recent years. By-products of ethanol production are often fibrous in nature and increased value for these products may be found through marketing as ruminant feedstuffs. Sweet sorghum silage serves as a multi-purpose crop for the production of energy (Reddy et al., 2005) and feed (Almodares et al., 1999; Fazaeli et al., 2006) due to the high

concentration of sugar in the stalks. Previous work by Murray et al. (2008) concluded that the stem plus grain of sweet sorghum resulted in greater yield of fermentable carbohydrates than typical fuel crops, such as corn to support ethanol production. The use of sweet sorghum silage for the production of ethanol produces a consistent, quality fibrous byproduct similar to the post-ethanol extraction sorghum silage used in this study providing opportunity for its use in feedlot diets as an alternative forage source.

Improved feed efficiency is an important profitability consideration for cattle feeders and steers fed SS had increased growing phase G:F due to numerically greater ADG but similar feed intakes across treatments when replacing brome hay. Though growing phase diets were balanced for DM inclusion of hay versus sorghum silage, samples collected during the 14 d titanium feeding period indicated differences in fiber content between treatments. Diet analysis of fiber components fed during the titanium feeding period (d 28 to 42) found the SS diet to have greater NDF and lesser ADF content compared to the CON diet. The slightly greater NDF content of the SS diet may have supported cellulolytic bacteria action more sufficiently; resulting in the improved digestibility of NDF by steers fed the SS diet. However, the ADF fraction of the SS diet was less than that of the CON diet and a 14% decrease in ADF digestibility was observed in the SS-fed steers. Lundy et al. (2015) noted similar patterns in NDF and ADF digestibility by lambs fed wet distiller's grains resulting from a secondary, cellulosic-based, fermentation. The authors hypothesized the decrease in ADF digestibility was in response to processing methods from which more readily available fiber components had been extracted for ethanol production and the resulting fiber fraction was less digestible to the ruminant.

Differences in the digestibility of NDF and ADF fractions of the forage suggest the hemicellulose in sorghum silage is more digestible than that found in the hay used in this trial.

Adewakun et al. (1989) noted that digestibility of NDF, ADF, and hemicellulose was greater in weanling beef steers receiving sweet sorghum silage when compared to fescue hay by approximately 17, 23, and 6%, respectively. In the present study, the improved digestion of NDF in SS-fed steers compared to CON-fed steers may be due to differences in maturity of the two forages at harvest or physical damage to cell walls that occurred during the extraction process for ethanol. Additionally, lesser DMI by SS-fed steers during the digestibility assessment period and the smaller particle size of the sorghum silage may have collectively supported greater attachment to fiber by bacteria and slightly slower passage rate resulting in improved digestibility of NDF.

The CP content of the diets were formulated to be similar across treatments; however, analysis of the diets during the titanium feeding period indicate slight differences in diet concentrations of CP with the CON diet containing a greater CP concentration than the SS diet. However, even with a lesser concentration of CP in the diet a 3% improvement in protein digestibility was noted for SS-fed steers. This difference could be explained by the inclusion of urea in the SS diet to meet requirements for CP as the sorghum silage had a lesser CP content than the hay. Adewakun (1989) hypothesized the greater sugar content of the sorghum silage contributed more readily available carbohydrates allowing for increased microbial protein production and CP digestibility observed in their study. Synchronization of N from supplemented urea in combination with readily available carbohydrates of the SS, may explain the increased CP digestibility by steers fed the SS diet observed in the present study.

Diet EE concentration was very similar across the two treatments; however, improved EE digestibility by SS-fed steers during the growing period may suggest differences in the fatty acid profile of the sorghum silage compared with the hay, as this may influence the site of digestion

and subsequent utilization by the animal. Alternatively, the difference could be due to plant physiology and the location of the fat within the respective forages utilized in this study. While oil content of hay was likely limited to the leaves as suggested by others (Boufaid, 2003; Palmquist, 2003), the post-extraction sorghum silage utilized in the present study did include small seeds which may have contained oil that was differentially available to animal following the ethanol extraction process.

Finishing diets used in the U.S beef industry generally contain high amounts of concentrates, with minimal amounts of roughage due to negative associative effects of the rumen microbiome and losses of efficiency as a result of energy dilution (Stock et al., 1990). In order to maintain rumen integrity in cattle fed typical finishing diets Owens (1987) suggests roughage inclusions from 5 to 15% of diet DM and common roughage sources include corn silage, corn stalks, and alfalfa hay (Samuelson et al., 2016). Galyean et al. (2003) suggests when comparing roughage sources in feedlot diets to balance for eNDF to assure rumen function as imbalances in the rumen microbiome can lead to metabolic disorders such as acidosis, resulting in decreased animal performance when steers are fed high concentrate diets (Fulton et al., 1979). Thus, to ensure proper rumen function finishing phase diets were formulated to achieve approximately 6% eNDF from each forage source. Concentrations of eNDF were approximately 46% eNDF for the hay, while the sorghum silage contained 37% eNDF. As a result of lesser eNDF dietary inclusions of SS were greater than that of the CON diet during the finishing period. Others have noted that incremental increases in roughage levels (0-24%) of feedlot diets tend to increase DMI (Gill et al. 1981; Kreikemeier et al., 1989). Similarly, Stock et al. (1990), reported when using a roughage blend (50% corn silage, 50% alfalfa silage) fed with different grain sources, that increasing roughage concentration from 0 to 9% numerically increased DMI and decreased feed

efficiency. Galyean and DeFoor (2003) report the increase in DMI is a result of energy dilution. The results of the present study contradict those of others, as steers receiving the SS diet consumed 1.3 kg less DM a day despite increased roughage in the diet suggesting the energy content of the SS is greater than that of the hay used in this study.

Overall trial performance indicates that SS-fed steers consume less feed but maintain similar growth and carcass composition when compared to steers fed grass hay. Inclusion of sorghum silage in growing and finishing diets produced comparable carcasses to that of CON-fed steers. Similar performance by steers despite additional post-extraction sorghum silage partially replacing cracked corn, the primary energy source in the diet, suggests the feeding value of sorghum silage is equivalent to or better than the average quality hay used in this study. However, the low forage inclusions used in this study are not optimal to measure energy value of a feedstuff. Thus, additional work is needed to quantify the energy value of this post-ethanol extraction sorghum silage when replacing corn in finishing diets. Results of this study suggest the energy density of post-extraction sorghum silage is equal to or greater than the hay used in this study, and dependent on availability provides opportunity for producers to utilize the consistent quality post-ethanol extraction sorghum silage as alternative forage in feedlot diets.

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TABLES AND GRAPHS

Table 1. Ingredient and chemical composition of diets fed to yearling steers (% DM basis)

	Growing diet		Finishing diet[1]	
Ingredients	CON[2]	SS[3]	CON[2]	SS[3]
Sorghum silage	-	40.0	-	16.0
Mixed grass hay, ground	40.0	-	13.1	-
Cracked corn	37.0	37.0	56.9	54.0
DDGS[4,5]	16.3	15.3	3.34	2.33
MDGS[6]	-	-	25.0	25.0
CCDS[7]	5.0	5.0	-	-
Trace mineral premix[8]	0.024	0.024	0.024	0.024
Limestone	1.21	1.46	1.46	1.52
Vitamin A premix[9]	0.10	0.11	0.11	0.11
Salt	0.31	0.31	0.31	0.31
Rumensin 90[10]	0.014	0.014	0.014	0.014
Urea	-	0.75	-	0.27
Calculated composition[11]				
CP	13.4	13.5	14.2	14.2
NDF	33.3	31.0	21.0	21.6
Ether extract	5.2	5.0	3.2	3.0

[1] During finishing period forages were added to contribute 6% eNDF from respective forages.
[2] CON: Mixed grass hay was roughage source.
[3] SS: Sorghum silage was roughage source.
[4] DDGS: Dried distillers grains plus solubles.
[5] During titanium feeding period titanium dioxide replaced DDGS at rate of: 0.00116% within CON, 0.00122% within SS diet.
[6] MDGS: Modified distillers grains plus solubles.
[7] CCDS: Condensed corn distillers solubles.
[8] Provided per kg of diet DM: 0.10 mg Co, 10 mg Cu, 20 mg Mn, 0.10 mg Se, 30 mg Zn, and 0.5 mg I.
[9] Vitamin A premix contained 4,400,000 IU Vitamin A/kg.
[10] To provide at least 200 mg^{-1}• $steer^{-1}$• day^{-1} of monensin.
[11] Composition calculated using Dairyland forage analysis and NRC (2000).

Table 2. Analyzed nutrient composition of growing phase diets fed to steers during titanium dioxide feeding period (d 28 to 42)

	Growing diet	
Nutrient composition, % DM	CON[1]	SS[2]
TDM[3]	78.3	56.3
OM	91.9	93.0
NDF	34.6	42.9
Hemicellulose	13.1	25.0
ADF	21.5	17.9
CP	15.6	13.7
Starch	20.8	29.5
Ether extract	3.69	3.67

[1] CON: Mixed grass hay was roughage source.
[2] SS: Sorghum silage was roughage source.
[3] TDM: True dry matter (105°C)

Table 3. Effect of post-extraction sorghum silage inclusion in feedlot diets on BW, average daily gain, and feed efficiency of yearling beef steers.

	CON[1]	SS[2]	SEM	*P* – value
Pens (*n*)	6	6		
Live performance[3]				
Growing period[5]				
Initial BW[4], kg	396	397	10.1	0.95
d 56 BW, kg	482	491	3.5	0.13
DMI, kg/d	11.23	11.40	0.259	0.66
ADG, kg/d	1.53	1.67	0.066	0.19
G:F	0.136	0.146	0.0027	0.04
Finishing period[6]				
Initial (d 77) BW, kg	520	522	4.0	0.69
Final BW, kg	638	633	4.9	0.55
DMI, kg/d	15.29	13.97	0.216	0.008
ADG, kg/d	2.11	1.98	0.051	0.15
G:F	0.138	0.142	0.0028	0.35
Overall (d 0-133)				
DMI, kg/d	13.16	12.60	0.210	0.12
ADG, kg/d	1.82	1.78	0.038	0.48
G:F	0.137	0.140	0.0014	0.20

[1] CON: Mixed grass hay was roughage source.
[2] SS: Sorghum silage was roughage source.
[3] A 4% pencil shrink was applied to live body weights.
[4] For analysis of initial BW block was not included in the model.
[5] Growing period: d 0 to 56 of trial.
[6] Finishing period: d 77 to 133 of trial.

Table 4. Evaluation of sorghum silage as a replacement for medium quality hay on carcass characteristics of beef steers.

	CON[1]	SS[2]	SEM	*P*- value
Pens (*n*)	6	6		
HCW, kg	396	396	3.2	0.91
Dressing percent	62.1	62.6	0.261	0.23
12^{th}- rib fat, cm	1.55	1.64	0.08	0.40
KPH[3], %	2.62	2.65	0.060	0.71
REA[4], cm^2	87.6	87.6	1.51	1.0
Yield grade	3.51	3.62	0.060	0.25
Marbling score[5]	479	480	13.63	0.97
Quality grade[6]	3.50	3.33	0.218	0.61

[1] CON: Mixed grass hay was roughage source.
[2] SS: Sorghum silage was roughage source.
[3]KPH: Kidney, pelvic, heart fat.
[4] REA: Ribeye area.
[5] Marbling scores: slight: 300, small: 400, modest: 500.
[6] Quality grade: 2: Select^{+}, 3: Choice^{-}, 4: Choice.

Table 5. Effect of post-extraction sorghum silage inclusion in growing diets on diet nutrient digestibility (d 28- 42) in beef feedlot steers.

	CON[1]	SS[2]	SEM	*P*- value
Steers (*n*)	12	12		
Nutrient digestibility, %				
DM	76.7	78.1	0.73	0.19
OM	78.1	80.2	0.81	0.09
NDF	70.5	73.1	0.71	0.02
Hemicellulose	64.6	82.4	0.82	< 0.0001
ADF	74.1	60.0	0.78	< 0.0001
CP	74.0	77.2	0.60	0.001
Starch	93.2	94.2	1.01	0.26
Ether extract	84.0	91.1	0.71	< 0.0001

[1] CON: Mixed grass hay was roughage source.
[2] SS: Sorghum silage was roughage source.

Table 6. Effect of post-extraction sorghum silage inclusion in growing diets on amount of nutrients digested (d 28 to 42) in beef feedlot steers.

	CON[1]	SS[2]	SEM	*P*-value
Steers (*n*)	12	12		
DMI, kg/d	12.05	11.17	0.188	0.003
Nutrients digested (kg)				
DM	9.24	8.72	0.165	0.04
OM	8.65	8.33	0.157	0.16
NDF	2.94	3.50	0.067	< 0.0001
Hemicellulose	0.98	2.30	0.044	< 0.0001
ADF	1.84	1.20	0.065	< 0.0001
CP	1.33	1.19	0.047	0.04
Starch	2.33	3.13	0.049	< 0.0001
Ether Extract	0.36	0.37	0.012	0.38

[1] CON: Mixed grass hay was roughage source.
[2] SS: Sorghum silage was roughage source.

CHAPTER 5.

GENERAL CONCLUSIONS

Sustainability of the beef industry is dependent on several factors, including the ability to continually utilize affordable fibrous by-products and meet consumer's preferences for beef, while being economically competitive with other protein sources. Profitability in the beef industry is impacted by the production costs associated with feed, which has prompted the beef industry to place emphasis on the need to improve feed efficiency (**FE**). Therefore, the studies presented in this thesis were designed to evaluate the implications FE may have on tenderness of beef and to evaluate the use of an alternative forage in feedlot diets.

The research project presented in Chapter 3 was developed from the conclusions of McDonagh et al. (2001), following a single generation of divergent selection for improved FE, as they found more efficient cattle had greater calpastatin activity, suggesting the potential to alter ultimate meat tenderness. Utilizing a subsample of steers selected as phenotypic extremes for growing phase RFI determination, the data presented in Chapter 3 on the influence of FE on meat tenderness attributes confirm that highly feed efficient steers tended to have greater calpastatin activity; however, indicators of post mortem proteolysis such as troponin-T degradation (from steaks aged 2 or 14 d) and calpain 1 autolysis were not different. Furthermore, overall meat tenderness was not impacted by growing phase FE classification as evidenced by the lack of differences in WBSF of steaks from steers considered to be lowly or highly FE. As differences in beef WBSF values are undiscernible in phenotypic extreme steers greater than 1 SD from a larger population mean it seems unlikely current selection pressure for FE will negatively impact consumers' perceptions of beef. An unexpected finding of the present study is the effect of diet type on meat tenderness

attributes. Steers grown on the roughage based growing diet (MU-Rough) produced steaks with a greater WBSF than steers grown on MU-Corn, thus producing a less tender steak. The greater fiber content of the by-product based finishing diet (ISU-Byp.) elicited a response for decreased d 14 troponin-T degradation, indicative of less protein degradation, though WBSF was not affected by ISU finishing diet. Additionally, preliminary data from this study evaluating the effects of finishing phase FE (G:F) on tenderness attributes suggest proteolytic activity may potentially be affected by performance immediately prior to harvest. In this analysis, proteolytic activity was altered by diet regimen (growing and finishing combination) within growing phase FE classifications. Contrary to all other diet combinations where finishing phase G:F had no effect on calpastatin and d 2 troponin-T degradation, lowly FE steers grown on MU-Corn, and finished on ISU- Byp demonstrated decreased calpastatin activity and increased d 2 troponin-T degradation as finishing phase FE (G:F) increased. If this response is found to be repeatable in a larger study this would suggest that lowly FE cattle finished on more fibrous diets such as those typical in the Midwest may produce a more tender product as G:F increases. Dietary influences on calpastatin activity are poorly understood and more work is needed to clarify this response. Interestingly, for HFE steers grown on MU-Rough and finished on ISU-Corn, d 14 troponin-T degradation decreased as G:F increased. These differences suggest high-fiber diets may have greater impact on the tenderness of meat than improvements in FE and further work is needed to characterize the influence of diet on in vivo muscle environment and subsequent impact on the proteolytic systems critical in protein metabolism in the beef animal.

Advances in commodity processing to support biofuel production and the utilization of residual products as cattle feedstuffs stimulated the work documented in Chapter 4, which

evaluated the effect of post-ethanol extraction sorghum silage as a forage source in growing and finishing diets on steer performance, carcass characteristics, and nutrient digestibility. In comparison to diets containing average quality grass hay, the use of post-ethanol extraction sorghum silage in growing phase diets resulted in increased total tract digestibility of neutral detergent fiber (**NDF**), hemicellulose, ether extract (**EE**), crude protein (**CP**) and organic matter (**OM**), although acid detergent fiber (**ADF**) digestibility was decreased in sorghum silage-fed steers. Finishing phase diets were formulated to contain similar concentrations of eNDF contributed by dietary roughage source. Because sorghum silage had a lesser amount of eNDF more displacement of corn occurred in the sorghum silage diet; however, despite this, carcass characteristics of steers fed sorghum silage were not different from steers with hay as the roughage source. Interestingly, overall trial performance demonstrated decreased DMI for steers receiving the sorghum silage diet, in combination with a numerically better G:F, providing potential for the use of post-extraction sorghum silage in feedlot diets to improve producer profitability. The differences in nutrient digestibility indicate the feeding value of post-ethanol extraction sorghum silage is better than that of average quality hay. The wide-spread use of sorghum silage will be dramatically influenced by geographical location of ethanol plants, as well as the economics of the ethanol industry as further advances and implementation of new processes are used in the processing of high-fiber feeds, like sorghum silage. Future research evaluating the feeding value of by-products, assigning values for energy, as well as an indicator of forage quality (NDF or eNDF), needs to be ready to assist producer's management decisions in order to effectively utilize these feedstuffs in growing and finishing feedlot diets to optimize cattle performance.

To date, research evaluating the relationship between RFI and meat tenderness on divergently selected animals has shown that as animals become more FE activity of calpastatin is increased, with the potential to negatively affect meat tenderness. The potential contribution of the calpain system, particularly calpastatin activity, to individual variation in FE between steers, may be minimal; however, more work is needed to elucidate why protein and fat accretion seems to be altered in cattle of varying FE classification. Although FE classifications have been reported to be repeatable across growing and finishing phases when diet type is similar, diets especially high in fiber often result in considerable variation in RFI among cattle. The use of high fiber diets may influence the proteolytic activity of the calpain system, subsequently altering postmortem protein degradation, decreasing meat tenderness. Further examination is needed to evaluate the effect of high fiber diets on changes in proteolytic activity of the calpain system *in vivo* and postmortem that elicit changes in FE and meat tenderness, more specifically the effect of finishing phase feed efficiency on this system. Collectively, advancing the understanding of relationships between diet type, FE, and proteolytic activity will aid in the nutritional management of cattle as the beef industry moves forward to remain economically competitive with other protein sources, while keeping its product on the plates of consumers.